Food Research

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VOLUME 21, NUMBER 5 SEPTEMBER - OCTOBER, 1956

An Official Publication of

The Institute of Food Technologists

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The Institute of Food Technologists

Volume 21 SEPTEMBER-OCTOBER, 1956 Number 5

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Published by the Institute of Food Technologists six times a year. Printed by The Garrard Press, 119-123 West Park Avenue, Champaign, Illinois. Entered as second-class matter at the Post Office at Champaign, Illinois, under the act of March 3, 1879. Accepted for mailing at special rate of postage provided for in amended Section 34.40(e), pursuant to the provisions of Public Law 233, approved October 8, 1951.

Food Technology

Published Monthly by the

INSTITUTE OF FOOD TECHNOLOGISTS

The Companion Journal to

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FOOD TECHNOLOGY

THE GARRARD PRESS

119-123 West Park Avenue

CHAMPAIGN, ILLINOIS

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To members of the Institute of Food Technologists, $7.50 per volume.

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Subscriptions and renewals are entered to begin with the first issue of the current volume.

Single copies of current volume: Domestic, $1.50; foreign, $1.75.

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INSIDE SCIENCE

The Vital Story of VITAMIN A

by Science Writer

We live in a golden age of the sciences!

In the science of nutrition discoveries of food elements and their action have resulted in the promise of better health for us all.

Let's consider vitamin A as an example.

A Capsule History. 1913 was the year. Two teams working independently with animal feeding experiments discovered that a diet which was good in all other respects but which varied only in the source of fat caused young animals to thrive or sicken as the fat was varied. The work of these teams (McCollum and Davis; Osborne and Mendel) led to the discovery of the food factor which we know as vitamin A.

Composition and Action. A pale yellow, oil-soluble substance, vitamin A is expressed in the chemist's shorthand as $C_{20}H_{29}OH$. It is necessary in the diets of men and animals to promote the body's growth and development. Vitamin A is a vital requirement in guarding the health of the eyes and skin, and for resistance against infection. It helps maintain the health of mucous membranes and other specialized epithelial and glandular tissues.

Vitamin A enters the body as a constituent of food. Or it may be created within the body when a precursor of vitamin A is eaten. A precursor is a food element with potential vitamin A *value* but no actual vitamin A *content*. For instance, many green leaves have a high vitamin A value but, paradoxically, contain no actual vitamin A. The body converts the precursor into the vitamin. The most common precursor of vitamin A is beta carotene, the natural yellow coloring of many foods, such as dairy products, cereal grains, carrots, alfalfa, etc.

When consumed in food vitamin A is absorbed primarily in the healthy intestine, is esterified in the intestine's walls and is carried as an ester in the lymph and blood to the body's storage depots. The liver, which has a large capacity for storage, contains about 90% of the vitamin A found in the body.

Isolation and Synthesis. It was not until 1937 that pure, crystalline vitamin A was isolated by Holmes and Corbett from fish-liver oil. Research in the synthesis of vitamin A was stimulated during the Second World War by the threat to natural sources in fish-liver oil. This work resulted in several processes to produce vitamin A synthetically. Of these, one of the most successful was evolved by the Roche research team headed by Dr. Otto Isler which was announced in 1947. Much of the vitamin A now used for pharmaceutical manufacturing and food fortification in the United States and many other countries of the world is produced by this process.

Vitamin A and beta carotene produced by the Hoffmann-La Roche synthesis have many positive advantages for food and pharmaceutical manufacturers. Among these are the complete absence of any "fishy" odor, taste, or after-taste; the reasonable and stable price; their stability, purity and uniformity and the assurance of a steady economical supply.

Vitamin A is described in the U. S. Pharmacopoeia as a fully acceptable compound for use in pharmaceutical products. The U. S. Food and Drug Administration has approved its use for the fortification of margarine and other foods.

Human Requirements. The Food & Nutrition Board of the National Research Council recommends that a daily allowance of 5000 International Units of vitamin A in the diet will maintain good nutrition of healthy adults in the U.S.A. The Board's recommendations vary above this for pregnant and lactating women, and below for infants and children. The adult dose used in therapy may vary from 50,000 to 200,000 units daily.

Production. At the huge Roche Park plant of Hoffmann-La Roche in Nutley, New Jersey, over 500 pieces of large-scale chemical engineering equipment are working constantly to produce the superior 'Roche' vitamin A. This production is so large that 'Roche' vitamin A comes from the plant actually by the tons. In fact, the entire needs of the food and pharmaceutical industries of the United States and Canada can be met from this one plant.

Again the Roche people, who have so often led the way in vitamin research and production, have made another contribution to the better health and well-being of countless millions.

This article is one of a series giving a brief resume of our present knowledge of those essential health factors —the vitamins. Reprints of this and other articles are available without charge. The Vital Story of Vitamin C was published previously and others are forthcoming. Send your request to: Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 286 St. Paul St., W.; Montreal, Quebec.

CHEMICAL CHANGES IN FISH ACTOMYOSIN DURING FREEZING AND STORAGE

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(Manuscript received March 13, 1956)

Following the current trend in food preservation, fishery products are being prepared and sold in the frozen state in increasing quantity each year. It is well known, however, that a decided consumer preference for fresh fish to frozen fish exists in many areas, despite the convenience of the frozen product. The reasons for this preference have been well recognized and have been the basis for extensive study throughout the world for many years.

The important changes *(2)* in fishery products that have been associated with freezing and cold storage are loss of tenderness and characteristic fresh-fish flavor. There is a subsequent development of texture changes—variously described as toughness, stringiness, mushiness, etc.—and of off-flavors and off-odors. Development of off-flavors and -odors is usually attributed to fat oxidation, even in lean fish. Loss of characteristic fresh-fish texture has been related to what is termed protein denaturation or, more simply, alteration.

Protein denaturation may be caused by many agents or conditions and, in effect, may result in a product of quite variable quality. Since relatively little is known concerning the true nature or structure of proteins, denaturation and its over-all effect on the quality of a food product is also incompletely understood at the present time.

Attempting to develop more objective methods for the measurement of frozen fish quality, Reay and Kuchel *(6)* applied to fish a procedure earlier used in classical studies on the proteins of rabbit muscle. Reay found a decrease in the solubility of the muscle proteins in salt solutions after frozen storage of the fish and drew attention *(7)* to the denaturation phenomenon. On further investigation, Canadian workers *(3)* showed that it was the protein fraction called actomyosin which was denatured. The non-actomyosin fractions remained unchanged except after very long storage. In combination with taste panel testing, it has been shown *(2)* that the solubility of actomyosin appears to parallel and to anticipate changes in texture and flavor, thus providing a measure of the quality of a particular sample of fish.

This paper reports a study of changes in properties of the actomyosin fraction of fish muscle that has been subjected to frozen storage. The properties of chemical activity, asymmetry, and solubility of the protein were investigated. An attempt has been made to gain a better understanding of the basic nature of the adverse effects of freezing and cold storage on the quality of fish protein.

EXPERIMENTAL

Materials and methods. All of the experiments reported in this paper were carried out on actomyosin isolated from the muscular tissue of yellow-striped rockfish *(Sebastodes nebulosus)*.

Preparations of actomyosin were isolated by a slight modification of the methods of Dyer, French, and Snow *(3)* and Greenstein and Edsall *(4)*. A 15.0 g. sample of finely ground flesh was extracted with 280 ml. of pH 7.30 buffer solution (0.50 N.KCl, 0.030 N. $NaHCO_3$) for 2 hours at 0-2° C. in a full, inverted, half-pint Ball Mason jar, using a Hamilton Beach blender connected through a powerstat to allow very slow stirring. This device completely eliminated foam formation by preventing a vortex in the swirling liquid while insuring good disintegration of the tissue. The resulting homogenate was then centrifuged for 30 minutes at 4240 R.C.F. at 0-2° C. The supernatant material was diluted with 9 volumes of 0° C. distilled water and allowed to stand at 0-2° C. for 16 hours. The actomyosin gel was removed by centrifuging for 30 minutes at 1350 R.C.F. at 0-2° C. and washed twice with 0° C. distilled water. Solid KCl was slowly added to the gel until a sol containing 0.50 N.KCl resulted. The resulting sol was clarified by centrifuging for 30 minutes at 4240 R.C.F. at 0-2° C. Protein concentration was estimated by the biuret method *(8)*, employing a Beckman spectrophotometer, model DU, at 5400 Å. The method was standardized by Kjeldahl nitrogen determinations *(5)*, using actomyosin solutions and a conversion factor of 16.6 *(4)*.

In order to characterize any change in chemical reactivity of the material in question, the total and "reactive" sulfhydryl (–SH) content of the isolated actomyosin was estimated by the method of Tsao and Bailey *(9)*.

Viscosity determinations were carried out with 5 ml. aliquots of various dilutions (with the pH 7.30, 0.50 N.KCl, 0.030 N. $NaHCO_3$ buffer solution) of the actomyosin preparations at 0.00 ± 0.02° C. using Ostwald viscometers calibrated with the same buffer solution.

Solubility of the actomyosin was estimated by comparing the total recovered actomyosin obtained during the usual extraction and precipitation process with the total protein content of the original 15.0 g. sample.

PROCEDURE AND RESULTS

Chemical activity (–SH tests). Actomyosin was isolated from fresh, post-*rigor* flesh and flesh that had been held at –18° C. for 6 months. The total and reactive –SH groups of the "salt solution-soluble fraction" from each sample were determined. The results of this experiment indicated that when actomyosin retains its salt solution-solubility, it shows very little change in reactive –SH groups and no change in total –SH groups, based on a titration accuracy of 5% (9). The total –SH (as g. cysteine/100 g. protein) and reactive –SH (groups reacting with N-ethylmaleimide at pH 7.3 as percentage of the total –SH) content of the actomyosin isolated from fresh muscle was 0.77 g. and 52.4% and from thawed muscle was 0.75 g. and 55.6%, respectively. Similar tests on the fraction which loses its salt solution-solubility met with less success, however. Actomyosin isolated from fresh, post-*rigor* flesh was frozen, thawed, and centrifuged to remove the residual soluble phase. Tests for reactive –SH groups on the aggregated actomyosin gave an indication of an increase (from 52.4 to 56-66%) in reactive –SH groups although the uncertainties inherent in the heterogeneous titrations precluded a positive result. Total –SH groups for the aggregated phase, measured by homogeneous titration in guanidine solution, showed no change (0.76 g.) from the soluble phase.

Asymmetry and solubility. Although the results of the sulfhydryl tests did not indicate a significant change in the structural configuration of the actomyosin prior to its loss of solubility, it was felt that a more subtle test might detect alteration in other characteristic properties prior to its aggregation. Viscosity determinations were thus carried out with dilute

(0.05-0.5%) solutions of actomyosin isolated from thawed muscle that had been subjected to increasing frozen storage periods.

The general procedure was as follows: Fillets from yellow-striped rockfish (filleted and skinned within 6 hours of capture—holding temperature approximately 7° C.—) were individually wrapped in aluminum foil and allowed to stand for 24 hours at 4° C. for the flesh to completely pass through *rigor*. The wrapped fillets were then frozen at –30° C. and held at –30° C. until removed for analysis. As needed, the wrapped fillets were allowed to thaw by placing them in a refrigerator (0-4° C.) for 16 hours. Viscosity and solubility determinations were carried out on the actomyosin isolated from each sample. The results of these tests, covering a period of 10 months' frozen storage, are given in Table 1.

TABLE 1

Intrinsic viscosity and solubility of actomyosin isolated from frozen-thawed yellow-striped rockfish as a function of storage time

Days storage of fillets at –30° C.	Intrinsic viscosity[1]	Soluble actomyosin[2]
0	1.70	48
¼	1.60	47
1½	1.17	52
8	1.10	49
14	1.22	57
21	1.12	54
28	1.08	60
42	1.10	55
63	0.95	50
91	1.18	48
206	1.25	29
300	1.20	24

[1] Viscosity increment at zero protein concentration (obtained by extrapolation when the viscosity increment is plotted against protein concentration).

[2] $\frac{\text{Total recovered actomyosin} \times 100}{\text{Total protein in 15.0 g. sample of muscle}}$

In order to accurately establish a "zero-time" or standard value for the viscosity of actomyosin from fresh, unfrozen muscle and the relative effect of prefrozen storage at 0° C., an experiment was carried out as follows: Several fish were filleted while alive and a fillet from each fish was wrapped in aluminum foil and held in crushed ice until withdrawn for analysis. An actomyosin preparation was initiated from a separate fillet at intervals of 1 hour, 1 day, 5 days, and 8 days after death of the fish. Viscosity determinations were carried out on the respective preparations after the same length of time had elapsed (5 days) after each preparation was initiated. Prelinimary work indicated that a very slight progressive decrease of viscosity of dilute actomyosin solutions occurred at 0° C. All operating and holding temperatures were as close to 0° C. as possible. The results of these determinations are given in Table 2.

From the results of these experiments (Tables 1 and 2), it would appear that actomyosin is quite stable in post-*rigor* flesh held at 0° C. for at least a week, yielding an average value of 1.70 for the intrinsic viscosity. The pre-*rigor* extracted protein, although certainly containing a fair amount of actomyosin, must be contaminated with actomyosin precursors, probably not myosin, since a value of 2.05 was obtained for

TABLE 2

Intrinsic viscosity of actomyosin isolated from unfrozen yellow-striped rockfish

Time after death of fish prior to actomyosin preparation[1]	Intrinsic viscosity[2]
1 hour	2.05
1 day	1.62
1 day	1.70
1 day	1.75
1 day	1.64
5 days	1.72
8 days	1.77

[1] Fillets held at 0° C.
[2] Viscosity increment at zero protein concentration (obtained by extrapolation when the viscosity increment is plotted against protein concentration).

the intrinsic viscosity of this preparation. When the flesh is frozen, although no early significant change in solubility of the actomyosin is noted, the viscosity shows an immediate decrease, reaching a minimum after only one and one-half days of frozen storage at –30° C. Although the actomyosin solubility then changed with frozen storage, the intrinsic viscosity of the isolated material remained quite constant at an average value of 1.14 with no apparent trends, until completion of the storage period (300 days). The actomyosin solubility showed an increasing trend toward a maximum solubility after about one month's storage and then declined quite continuously beyond that point.

DISCUSSION

The conclusions from this study are that: (1) The freezing process itself produces some subtle change in the structure of actomyosin present in post-*rigor* fish muscle, yielding a more symmetrical molecule. A significant change in structural configuration was not indicated by the very slight increase in reactive –SH groups, however. (2) Continued frozen storage is responsible for some yet unknown change which causes progressive and irreversible aggregation of the actomyosin, although no indication of a "preliminary step" or trend prior to aggregation was detected. (3) When actomyosin "freeze-denatures" or aggregates, changes in structural configuration probably occur. It would appear that the salt-insoluble or aggregated fraction is that which is most closely related to texture changes associated with cold storage fish. Due to the nature of the aggregated protein, attempts to study this fraction have thus far met with little success.

Addendum. Practical storage tests by Dyer and collaborators *(2)* definitely indicate a variable cold storage life for different species of fish which is related to the degree of actomyosin aggregation. Dyer *(1)* states that a comparison of the cold storage stability of different species suggests a relationship to lipid content. Recent unpublished work at this laboratory would support this finding, suggesting a possible correlation between cold storage stability and the lipid complexed with the actomyosin of various species of fish.

SUMMARY

A study was made of the changes in sulfhydryl (–SH) content, viscosity, and solubility properties of actomyosin isolated from frozen fish.

When actomyosin was frozen and thawed, that fraction still soluble in dilute salt solution showed only a very slight increase in reactive –SH groups (from 52.4 to 55.6, as percentage of the total –SH groups). That fraction which aggregated, or lost its salt solution-solubility, showed a larger but variable increase (from 52.4 to 55-66%). Total –SH groups remained constant on freezing (0.76 g. cysteine per 100 g. protein).

On freezing actomyosin, an immediate decrease of the intrinsic viscosity was noted (1.70 to 1.17). On prolonged frozen storage (–30° C. for 300 days), no further significant change in the intrinsic viscosity was observed.

Actomyosin solubility showed an increasing trend toward a maximum solubility after about one month's storage and then declined quite continuously beyond that point.

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PENETRATION AND GROWTH OF *SALMONELLA* IN SHELL EGGS

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(Manuscript received March 24, 1956)

There are several ways in which shell eggs can become contaminated, externally and internally, with *Salmonella*. Normally, the oviduct of the hen is sterile and therefore the shell and internal contents of the egg are also free of microorganisms *(10, 16)*. In some instances, however, the ovaries and oviduct may be infected with *Salmonella* and these may be deposited inside the egg *(12)*. More frequently, however, the egg becomes contaminated after it is laid. *Salmonella* are deposited on the shell from the intestinal tract of the hen and from other sources in the environment *(7)*. In one survey, 2% of clean eggs and 16% of dirty eggs were found to contain *Salmonella* on the surface or in the pores of the shell *(13)*. Shell contamination is undoubtedly a major source of *Salmonella* in dried and frozen liquid whole egg, yolk, and albumen. Moreover, these percentages of *Salmonella* are minimal figures due to inaccuracies inherent in the isolation and identification procedures *(9, 15)*.

The *Salmonella* cells in the pores of the shell are walled off from the egg contents by the shell membranes. The latter behave as mechanical rather than bactericidal barriers *(14)*. But under suitable conditions of temperature and moisture, the membranes can be breeched by *Salmonella*, by mechanisms which are as yet unknown, and this leads, eventually, to extensive bacterial multiplication in the highly nutritious albumen and yolk. Scott *(12)* readily infected both hen and duck eggs by immersing them in cultures of *Salmonella aertrycke* for a few days. If the culture was simply allowed to dry on the eggs, infection did not occur. Likewise Brown, Combs, and Wright *(2)* obtained infection of duck eggs by wrapping them in filter paper moistened with a broth culture of *S. aertrycke*.

Although the factors governing the penetration of *Pseudomonas* and other saprophytic bacteria into shell eggs have been investigated extensively under carefully controlled conditions *(4, 6, 8)*, similar studies have not been made with *Salmonella* despite their obvious importance. In the present investigations, the rate and extent of *Salmonella* infection of eggs have been determined as functions of type of *Salmonella*, cell concentration, and incubation temperature.

METHODS

The *Salmonella* strains included *S. oranienburg* 200 E, *S. montevideo* 140 E, *S. typhimurium* Tm-1, *S. gallinarum* 222, and *S. pullorum* 1431. The latter two nonmotile organisms served to indicate whether motility influenced penetration. Clean, fresh, grade AA, hen's eggs were exposed to infection by the same technique used so effectively with *Pseudomonas* by Lorenz *et al. (8)*; the method involves immersion of warm eggs in cold bacterial suspensions and causes the bacteria to be drawn through the pores to the membranes. The suspensions of *Salmonella* were prepared by removing the cells from one-day-old trypticase soy agar plate cultures with small amounts of sterile distilled water. These were diluted further with sufficient water to give a

reading of 50 in the Klett-Summerson photometer (red filter). Such suspensions contained approximately 4×10^8 cells per ml.; the exact bacterial numbers were obtained by dilution and plating with trypticase soy agar.

To infect the eggs, the cell suspensions were cooled to 4 to 5° C. in ice water and the eggs were warmed by incubation at 35° C. for several hours. The eggs were then completely immersed in the cold bacterial suspensions for 30 minutes, removed, air dried briefly at 35° C., and then stored either at 29° C. and a relative humidity of approximately 65% or at 1° C. and a relative humidity of about 80%. At intervals, some of the eggs were removed for analysis.

To determine whether the *Salmonella* had penetrated into an egg, the egg was immersed in 70% ethanol for about 2 minutes and then flamed to destroy organisms in the shell. The egg was broken open, the contents allowed to drain out, and the albumen and yolk thoroughly mixed. Ten ml. of the mixture was plated with trypticase soy agar to determine total bacterial numbers and also a loopful was streaked on brilliant green agar to verify the presence of *Salmonella*. Colonies from the latter medium were examined for typical appearance, reddening of the surrounding medium, and agglutinability with specific group antiserum. Colonies on the trypticase soy agar plates were also examined and occasionally tested for agglutinability with *Salmonella* antiserum. Penetration was considered to have occurred unequivocally only when positive brilliant-green agar cultures were accompanied by counts of at least several hundred cells per ml. of egg material with trypticase soy agar. In this way positive results, due possibly to chance contamination of the egg contents by a few surviving *Salmonella* cells in the shell, were eliminated from consideration. Controls included untreated eggs and also warmed eggs immersed in cold distilled water. Aseptic precautions were employed in all operations and there was no evidence at any time of any significant extraneous microbial contamination. Additional details of technique will be presented later as needed.

INFLUENCE OF TYPE OF SALMONELLA

In the manner already described, several batches of eggs were immersed in aqueous suspensions of 5 different species of *Salmonella*. The concentrations of bacteria per ml. of suspension, as determined by plate counts, were as follows:

S. oranienburg	384,000,000
S. typhimurium	448,000,000
S. montevideo	459,000,000
S. pullorum	523,000,000
S. gallinarum	448,000,000

The eggs were then incubated at 29° C. and at intervals ranging from 0 to 29 days, an egg from each batch treated with a particular *Salmonella* strain was analyzed, qualitatively and quantitatively, for internal content of *Salmonella*. The results are given in Table 1.

Data for the untreated control eggs and those immersed in water are not presented in the table, but these eggs were consistently negative for *Salmonella* or other bacteria at all sampling periods. This fact virtually eliminates the possibility that the eggs used in the experiment were initially naturally infected internally with *Salmonella* or later with other bacteria and permits interpretation of the results entirely on the basis of the experimentally introduced *Salmonella*. Data in Table 1 present several interesting aspects. The absence of *Salmonella* within any of the eggs immediately after treatment, *i.e.*, at zero time, indicates that the method used to sterilize the shell was effective although not necessarily completely so, as suggested below. Moreover, it indicates that during immersion, the

TABLE 1

Penetration and growth of various Salmonella strains in shell eggs at 29° C.

Days of incubation	Medium	*S. oranienburg*	*S. typhimurium*	*S. montevideo*	*S. pullorum*	*S. gallinarum*
0	BGA[1]	−	−	−	−	−
	TSA[2]	0	0	0	0	0
5	BGA	−	−	++++	+++	−
	TSA	0	0	23,000	1,900	0
15	BGA	−	−	+	−	+
	TSA	0	0	25	0	10
22	BGA	−	++++	++++	++++	−
	TSA	0	29,600	100,000	100,000	0
26	BGA	++++	++++	++++	−	++++
	TSA	>10,000,000	>10,000,000	> 10,000,000	0	>10,000,000
29	BGA	++++	−	++++	++++	++++
	TSA	710,000,000	0	1,350,000,000	630,000,000	50,000

[1] Brilliant-green agar; − indicates no growth and + to ++++ indicates increasing amount of growth.
[2] Trypticase soy agar; the figures represent the number of *Salmonella* per ml. of albumen-yolk mixture.

Salmonella cells were sucked to but not through the shell membranes. After 15 days of incubation, *Salmonella* cells were found, apparently internally, in an occasional egg from some batches; penetration appears to have taken place. In these instances, however, the numbers of cells were considerably less than 100 per ml., and this fact suggests the possibility of manipulative contamination of the egg contents by residual *Salmonella* in the incompletely sterilized shell. However, the thousands of *Salmonella* cells recovered after 5 days from the eggs treated with *S. montevideo* and *S. pullorum* preclude such shell contamination and indicate penetration of the *Salmonella* through the membranes and into the egg contents.

Most important is the fact that, after 3 to 4 weeks, all 5 strains of *Salmonella* had not only penetrated, without question, into the egg contents but also had multiplied there to enormous numbers which approached or exceeded a billion cells per ml. of albumen-yolk mixture.

The occurrence of eggs free of *Salmonella* after large numbers had been found in the particular batch at a previous sampling period underlines the known variability between individual eggs. The greater degree of resistance of a particular egg to *Salmonella* penetration may be due to a lesser porosity of the shell, to possession of a thicker or more dense membrane, or to a greater content of anti-bacterial substances such as lysozyme, avidin, and conalbumin in the albumen.

There was excellent correlation between the qualitative and semi-quantitative results on brilliant green agar and the quantitative data on trypticase soy agar. Invariably, positive and luxuriant growth on the former was accompanied by high counts on the latter. The *Salmonella* cultures isolated from the internal contents of the eggs corresponded in appearance and agglutinability with antiserum to those with which the eggs were originally treated.

The possibility that the total counts may include some non-*Salmonella* contaminants originally in the shells and which penetrated the eggs cannot be entirely excluded. But this possibility seems remote in view of the sterility of the internal contents of the two sets of control eggs, untreated, and especially those immersed in water alone, at all sampling periods. Also, the good agreement between amount of growth on brilliant-green agar and total counts minimizes the possibility of extraneous contamination.

The results also suggest that non-motile strains, *S. pullorum* and *S. gallinarum*, can penetrate eggs as readily as motile *Salmonella* strains. This is not surprising in view of the structure of the protective shell membranes. These are composed of dense layers of interlacing keratin fibers, the interstices of which are plugged with albuminous material *(11)*. It would seem more likely, therefore, that the enzymatic, probably proteolytic, activities of the bacteria would be of far greater importance for penetration of the membranes than cell motility.

EFFECT OF CELL CONCENTRATION AND TEMPERATURE OF INCUBATION

A suspension of *S. oranienburg* containing 4×10^8 cells per ml. was prepared. Aliquots were diluted 10^2, 10^4, and 10^7 with water to give suspensions containing, respectively, 4,000,000, 40,000, and 40 cells per ml. These calculated values were confirmed by plate counts. Batches of eggs were treated in the usual manner with the four decreasing concentrations of cells. One set of eggs was incubated at 29° C. and a duplicate set at 1° C. The latter temperature was chosen to provide information on *Salmonella* penetration under cold storage conditions. At intervals up to 1 month at 29° C. and almost 6 months at 1° C., usually 4 replicate eggs from each treatment were assayed for internal *Salmonella*.

TABLE 2

Effect of cell concentration on penetration of shell eggs by Salmonella oranienburg at 29° C.

Days of incubation	Egg assayed	Cells per ml. of suspension			
		4×10^8	4×10^6	4×10^4	4×10^1
		Cells per ml. of egg material			
7	1	10	0	0	0
	2	10	0	0	0
	3	10	>100,000	40	0
	4	0	0	0	0
16	1	30	110	0	0
	2	1860	16,200	0	0
	3	3400	105,000	51,000	100,000
	4	$>1 \times 10^6$	$>1 \times 10^6$	96,000	370,000
21	1	347×10^6	0	0	0
	2	521×10^6	$>10 \times 10^6$	4,800	0
	3	654×10^6	$>10 \times 10^6$	$>10 \times 10^6$	0
	4	684×10^6	$>10 \times 10^6$	$>10 \times 10^6$	10
28	1		825×10^6	66,000	0
	2		$>1000 \times 10^6$	614×10^6	0
	3			652×10^6	0
	4				300,000

Results at 29° C. The data are presented in Table 2. Major steps in the infection process are graphically outlined by the results obtained with the highest cell concentration, 4×10^8 cells. If one excludes shell contamination as a possible complicating factor, a week is required for the *Salmonella* cells to penetrate the shell membranes and appear within the egg. At this time the small number of only 10 cells per ml. of egg could be found. During the next week, these few successful invaders apparently gradually overcame the defensive mechanisms within the egg and in the process multiplied to somewhat larger populations, in the order of several thousand cells per ml. Finally, during the third week, when the breakthrough had been consolidated, bacterial growth increased enormously to numbers of about one-half billion cells per ml. of egg melange. Thus the successive steps of *Salmonella* infection appear to be those of a relatively slow penetration, followed by a period of slow growth and finally by a period of rapid multiplication to a very large number of *Salmonella*.

Results similar to those above were obtained with the eggs immersed in the suspensions containing 4×10^6 and 4×10^4 cells per ml. However, with the low number of 40 cells per ml. most of the eggs were not infected. Nevertheless, the fact that penetration and growth of *Salmonella* did occur with some of the eggs in this series is of considerable practical significance because it indicates that infection of eggs can be brought about even by small numbers of *Salmonella*. The latter point receives support from some of the data obtained from the 1° C. experiment as indicated below.

The same types of controls were used in this experiment as in the previous one. The untreated eggs and those immersed in sterile water were negative for *Salmonella* or other bacteria at each sampling period. Colonies that developed on the brilliant-green agar plates exhibited the characteristic properties of *S. oranienburg*. These results provide evidence that all of the data concern only the fate, in the eggs, of the experimentally introduced *Salmonella* cells.

Results at 1° C. The duplicate set of eggs which were stored at 1° C. was sampled after 20, 50, 128, and 168 days. Results were strikingly different from those at 29° C. In no instance could *Salmonella* be isolated from the contents of any of the eggs treated with the different bacterial concentrations; the egg contents were sterile. Obviously, *Salmonella* cells which had been sucked into the pores of the eggs and against the shell membranes could not penetrate the latter at 1° C.

To determine whether the shell and membranes still harbored the inoculated *Salmonella*, after 168 days, two types of tests were made. In one, an egg from each of the 4 cell concentration groups was broken out without sterilizing the shell. The egg contents were discarded and the shell and membranes were disintegrated in a mechanical blender with 50 ml. of sterile water for 2 minutes. Four loopfuls of the blended material were streaked on brilliant-green agar. Also, 10 ml. was pipetted into selenite F broth for enrichment of the possibly few cells which might be present and could be missed by the method of streaking directly on brilliant green agar. After overnight incubation the enrichment cultures were streaked on brilliant-green agar.

Results are presented in Table 3. The plates which were streaked directly from the blended shell material were all negative for *Salmonella*.

TABLE 3
Survival of Salmonella oranienburg in the shell and membranes of eggs stored for 6 months at 1° C.

Cells per ml. immersion fluid	Recovery of *S. oranienburg* from shell and membranes		Internal infection on incubation at 29° C.
	Direct	After enrichment[1]	
4×10^8	−	+	+
4×10^6	−	−	+
4×10^4	−	−	−
4×10^1	−	−	−

[1] Symbols: — no growth, + growth.

From the enrichment cultures, *S. oranienburg* was recovered only from the egg originally treated with the highest cell concentration (4×10^8 cells). In this instance, the negative results on direct plating and the positive results on enrichment indicate that there were only a few *Salmonella* cells in the shell. All of the results clearly establish that the *Salmonella* cells originally introduced into the shells of the eggs not only failed to penetrate the membranes at 1° C. but also virtually completely died out during the 6 months' storage at this low temperature.

This conclusion is supported by data obtained in the second type of test. Two eggs from each of the 4 cell concentration groups at 1° C. were removed and placed at 29° C. On the basis of the previous results at 29° C. it could be predicted that if viable *S. oranienburg* cells were present in the shells, internal infection would occur after about 3 weeks. The eggs were broken out and the contents were cultured after 5 weeks of incubation at 29° C. Large numbers of *Salmonella* cells were isolated from the eggs which had been in contact with the two highest concentrations of *S. oranienburg* but none from the eggs treated with the two lowest cell concentrations (Table 3). Infection in the eggs treated at 4×10^6 cell level is in contrast to the negative results of the enrichment experiment with the shell and membranes of similarly treated eggs. The difference may be due to variations in individual eggs or to use of only one-fifth of the total shell for enrichment and the entire shell in the incubation experiment. If only a few *Salmonella* cells survived in these eggs, as seems to be the case, then the incubation technique would offer the greater opportunity to detect them.

In general, however, there is good agreement between the results of the two techniques. The demonstration, by direct culture, that only a few cells survive in the shells of some of the eggs at 1° C. and that such eggs undergo infection on incubation at a more suitable temperature underlines again the fact that even a few *Salmonella* cells in the shell can infect an egg internally.

EFFECT OF TEMPERATURE ON GROWTH OF S. ORANIENBURG

The absence of infection in eggs treated with *S. oranienburg* and stored at 1° C. is in sharp contrast to the relatively rapid and extensive invasion of eggs by *Pseudomonas* and other bacteria at this low temperature *(6)*. The simplest explanation for this marked difference would seem to be that *Salmonella*, or at least *S. oranienburg*, cannot grow at 1° C., whereas pseudomonads and certain other bacteria can multiply at this

temperature. Gibbons, Moore, and Fulton *(5)* have reported that several strains of *Salmonella* produced little or no growth in liquid egg within 2 days at temperatures below 15.6° C. To test this explanation experimentally, cultures of *S. oranienburg* were streaked on trypticase soy agar plates and incubated at 1° C. and also at a number of higher temperatures in order to establish precisely the minimum growth temperature. A solid medium was used rather than broth in order to more closely approach the environmental conditions of the egg shell and membranes. Actually, subsequent experiments with broth cultures gave essentially the same results as those with the agar medium. For comparison, agar plate cultures of *Pseudomonas fluorescens* and *Pseudomonas ovalis*, both of which were isolated originally from rotted shell eggs, were also incubated at 1° C. The effect of temperature on growth of *S. oranienburg* is shown in Table 4.

TABLE 4

Growth of Salmonella oranienburg on trypticase soy agar at various temperatures

Temperature (° C.)	Growth in 1 day[1]	Comment
1	−	No growth in 1 month
5	−	No growth in 1 month
10	−	Slight growth in 1 month
15	±	Good growth in 1 week
20	+	Good growth in 2 days
29	++	
35	+++	
40	++++	
43	+++	
46	+	

[1] Symbols: − no growth, ± trace of growth, + to ++++ increasing amount of growth.

It is clear that *S. oranienburg* cannot develop at 1° C. and that the minimum temperature for growth is considerably above that temperature. Not the slightest trace of growth appeared on the plates placed at 1° C. and 5° C. even when incubated for as long as a month. These negative plates, however, developed luxuriant growth of *Salmonella* when removed from 1° C. after a month and placed at 35° C. for one day. The low temperatures repress growth of *Salmonella* but permit survival of many of the cells for at least a month. At 10° C. only slight growth appeared in a month. Thus the minimum temperature for growth of *S. oranienburg* is close to 10° C. Above 10° C., growth increases with increase in temperature up to 40° C. and then decreases rapidly as the temperature is raised beyond this point. The minimum, optimum, and maximum growth temperatures for *S. oranienburg* are therefore, respectively, 10° C., 40° C., and 46° C. Closely similar temperature ranges for growth have been obtained with many other *Salmonella* strains and appear to be characteristic for these organisms. Results of these more extensive studies will be published elsewhere.

There can be little question, therefore, that the inability of *S. oranienburg* (and this will undoubtedly be true of other *Salmonella* strains) to penetrate shell eggs stored at 1° C. is due to the inability of the organism to grow at this low temperature. Unable to multiply, the *Salmonella* cells gradually die so that externally contaminated eggs may be entirely free

of *Salmonella* after 6 months' storage, provided the initial contamination is not too great.

In contrast to the *Salmonella*, both strains of *Pseudomonas* formed numerous and large colonies on plates stored at 1° C. within a few days.

DISCUSSION

Relatively few studies have been made of the natural incidence of *Salmonella* in shell eggs, probably because of the very large amount of laboratory work required to analyze a significant number of eggs. Those that have been made with hen's eggs indicate that the extent of internal *Salmonella* infection is low and ranges from none in clean eggs *(1, 13)* to about 1.2 to 3.6% in dirty eggs *(3, 13)*. The latter percentage although low may be of considerable public health significance. Additional investigations are needed to establish more firmly the incidence of *Salmonella* in shell eggs.

However, as pointed out in the introduction, there is a much higher incidence of *Salmonella* on or in the shells of eggs than is found internally. The present investigations have shown clearly that at an appropriate temperature, *Salmonella* in the shell can penetrate the protective shell membranes and multiply to enormous numbers within the egg. Moreover, the data indicate that the initial degree of shell contamination need not be very large, probably less than 100 *Salmonella* cells, for infection to occur. Perhaps the greatest danger from this mode of infection lies in the possibility that a single shell egg, heavily infected with *Salmonella*, can contaminate large batches of sound eggs, when broken out to produce liquid, frozen, or dried whole egg, albumen, or yolk.

The data suggest also that shell eggs which are to be stored for several weeks or longer should be kept at temperatures below 10° C. to avoid penetration and growth of *Salmonella*. Storage at low temperatures appears to have the advantage not only of inhibiting growth of *Salmonella*, but also of leading eventually to their destruction. In this way infections of eggs with *Salmonella* can be controlled much more readily than those due to pseudomonads, since the latter organisms multiply rapidly even at 1° C.

SUMMARY

Shell eggs were contaminated with 5 strains of *Salmonella*, *S. oranienburg*, *S. montevideo*, *S. typhimurium*, *S. gallinarum*, and *S. pullorum* by immersing warm eggs in cold aqueous bacterial suspensions. When the contaminated eggs were incubated at 29° C. for 3 to 4 weeks, all of the strains penetrated the shell membranes and multiplied within the eggs to populations as high as one billion cells per ml. of egg meat. Penetration and growth occurred just as readily with the non-motile strains, *S. gallinarum* and *S. pullorum*, as with the motile strains.

The course of infection, as determined with *S. oranienburg*, consists of a period of relatively slow penetration of the shell membranes during the first week of incubation, followed by slow bacterial multiplication within the egg during the second week and finally by rapid growth to very large numbers during the third week.

Approximately the same rate and extent of penetration and growth of *Salmonella* in eggs was obtained with 4×10^8, 4×10^6, and 4×10^4

cells of *S. oranienburg* per ml. of immersion fluid. However, with the low concentration of 4×10^1 cells only a portion of the eggs became infected.

In sharp contrast to the occurrence of internal infection within a month in eggs contaminated with *S. oranienburg* and stored at 29° C., there was no sign of infection in identically treated eggs stored at 1° C. for as long as 6 months. Moreover, the inoculated *Salmonella* cells died out to a considerable extent during this relatively long incubation period at 1° C.

Absence of infection in the eggs stored at 1° C. is due to the inability of *S. oranienburg* to grow at this low temperature. Minimum temperature for the growth of this and other *Salmonella* strains is approximately 10° C. In contrast, *Pseudomonas* strains grow well at 1° C.

The data suggest that shell eggs which must be stored for several weeks or longer should be kept below 10° C. to prevent possible infection with *Salmonella*.

Acknowledgment

We are indebted to Mr. R. Paul Elliott of the U. S. Food and Drug Administration for providing us with the cultures of *Ps. fluorescens* and *Ps. ovalis*.

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THE ROLE OF DEHYDROASCORBIC AND DEHYDROREDUCTIC ACIDS IN THE BROWNING REACTION[a]

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The non-enzymatic browning reaction in foods has been the subject of a large amount of discussion and work in recent years. This reaction refers to the formation of brown colored substances in foods causing the product to become brownish to black in appearance and is usually characterized by undesirable changes in flavor, odor, and nutritive value. In addition the browning reaction in foods is often accompanied by an increase in acidity, reducing properties, and fluorescence.

Various theories have been advanced to explain the mechanism of browning *(5)*. Among these, the following are the most generally accepted: (a) *The Maillard or melanoidin condensation theory.* According to this theory—the most popular one—the reaction involves a condensation of amino acids and reducing sugars and gives rise to the formation of dark colored substances. (b) *The ascorbic acid theory.* According to this theory, the most important precursors to browning are ascorbic acid and related compounds which upon oxidation yield reactive products that may polymerize or react with nitrogenous constituents of the food to form brown pigments. There is a considerable amount of evidence in support of this theory so far as citrus products are concerned. (c) *The "active-aldehyde" theory.* In reality, this most recent of the theories is a modification of the other two. It is postulated that browning involves the decomposition of sugars and sugar acids to furfuraldehydes or similar compounds characterized by having an active carbonyl group, and that these products condense with nitrogen compounds and/or polymerize to form brown resinous materials.

In regard to the ascorbic acid theory of browning, Stadtman points out that browning in citrus fruit is always associated with a destruction of ascorbic acid. This fact has led to the suggestion that ascorbic acid is involved in the browning reaction in one of two ways: (a) It may act as an antioxidant, being oxidized in preference to other substances present which upon oxidation yield dark compounds or precursors of dark compounds. (b) The oxidation products of ascorbic acid may themselves be the actual precursors of dark compounds. One significant observation by Koppanyi *et al. (2)* is that dehydroascorbic acid reacts very rapidly with alpha amino acids to produce strongly colored (reddish to brown) complexes. The color reaction is apparently specific for the oxidized form of

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ascorbic acid. In view of the fact that browning in citrus products is preceded by an oxidation of ascorbic acid, the above suggests a possible mechanism for the browning of these products.

The following work was undertaken in an attempt to elucidate the role of ascorbic acid and reductic acid in the browning reaction. Reductic acid was included in this study because of its similarity, both chemical and physical, to ascorbic acid and because of its inclusion in "apparent ascorbic acid" compounds in heat-processed or stored foods *(6)*.

EXPERIMENTAL

The reductic acid used was prepared by the acid hydrolysis of citrus pectin, and is essentially that described by Reichstein and Oppenauer *(4)*. Purification was accomplished by recrystallization from ethyl alcohol-acetone (2:1) mixtures. The ascorbic acid employed was a U.S.P. grade of fine crystals.

The dehydro derivatives of both reductic and ascorbic acids were prepared by bromine oxidation. The bromine was slowly added to solutions of these compounds in N/100 HCl, drop by drop, while agitating the solution with a stream of washed nitrogen. The bromine addition was continued until a slight excess was indicated by the solution turning a light yellow color. Aeration was continued to remove the excess bromine.

RESULTS

In a series of experiments in this laboratory with both reductic and ascorbic acids, it was observed that a solution of dehydroreductic acid in N/100 hydrochloric acid lost its typical ultraviolet absorption after 2 days storage in the refrigerator at 4° C. and assumed instead a curve identical to that of reductic acid. Figure 1 demonstrates some typical curves for reductic and dehydroreductic acids. To investigate this point further, a solution of reductic acid, 10mM in 200 ml. of N/100 hydrochloric acid, was prepared and oxidized with bromine as described above. Samples were taken for titration of acidity, for spectrophotometric examination at 263 mμ wave length, and for titration with iodine, both before and after treatment with hydrogen sulfide. The hydrogen sulfide treatment was intended to determine the total reductic acid content of the solution by conversion of the reversibly oxidized form, dehydroreductic acid, to the reduced state, reductic acid. The remainder of the solution was stored at room temperature in a special glass-stoppered flask in an atmosphere of nitrogen. Similar examinations were made periodically. The nitrogen atmosphere was renewed at each examination. Results are shown in Table 1.

TABLE 1

Formation of reductic acid and increase in acidity in an acid solution of dehydroreductic acid held at room temperature

Time in days	Iodine titration				Spectrophotometric, 263 mμ		Increase in acidity ml. *N* acid per liter
	Total by H_2S		Direct (free)				
	mg./l	mM	mg./l	mM	mg./l	mM	
0	5700	50.0	0	....	0	....	....
2	5678	49.8	261.2	2.3	262.9	2.3	2.3
4	5487	48.1	587.1	5.2	577.0	5.1	....
7	4922	43.2	875.0	7.7	897.4	7.9	10.3
14	4004	35.1	1165.0	10.2	1181.4	10.4	20.3
21	3240	28.4	1367.0	12.0	1392.1	12.2	24.3

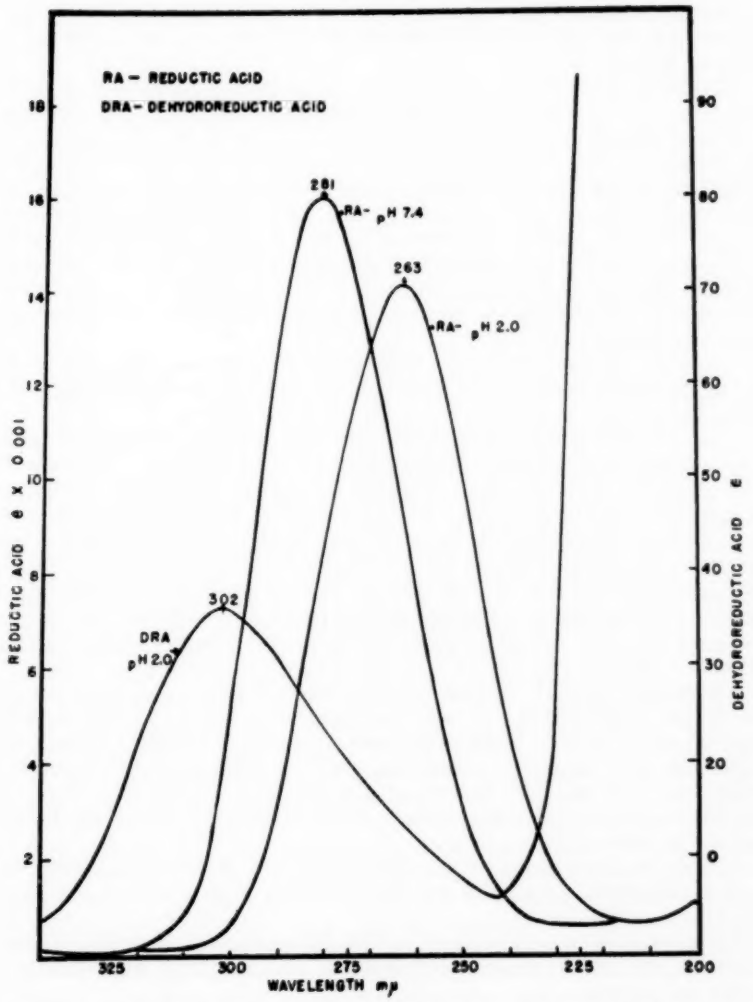


Figure 1. Ultraviolet absorption curves of reductic acid at pH 7.4 and 2.0, and of dehydroreductic acid at pH 2.0.

The results indicate the formation and subsequent increase of reducing substances as measured by iodine titration. Spectrophotometric examination of the solution in the ultraviolet region indicated an initial curve typical of dehydroreductic acid but the second, and subsequent, examinations revealed a curve identical with that of reductic acid. Calculation of the spectrophotometric readings in terms of reductic acid and comparison of these values with those obtained by direct iodine titration

demonstrates almost identical values. These results substantiate the initial observation that the reducing substance formed under these conditions is actually reductic acid.

Examination of the results obtained for the total reductic acid content, as measured by iodine titration after hydrogen sulfide treatment, indicates a loss of reductic acid. This loss in total reductic acid can possibly be explained as a loss due to oxidation of the compound beyond the dehydro stage. Oxidation may be compensated for by a simultaneous reduction of the dehydroreductic acid to reductic acid. That the dehydroreductic acid may be further oxidized is indicated by the concomitant increase of total acidity as measured by titration with standard alkali. It was also observed that a faint brownish discoloration of the dehydroreductic acid solution was observed on the fourth day and appreciable browning was evident by the twenty-first.

Another experiment was set up to further investigate this reaction at an approximate physiological pH, 7.25, and under thermostatically controlled conditions at 25° C.

A solution of reductic acid, 5mM in 100 ml. of N/100 hydrochloric acid, was prepared and carefully oxidized with bromine as above. The solution was allowed to come to temperature equilibrium in a carefully controlled water-bath maintained at 25 ± .05° C. Forty ml. of this dehydroreductic acid solution, in two 20 ml. pipettes, was added to a cylinder containing 120 ml. of water and 40 ml. of 0.5M Na_2HPO_4, also maintained at 25° C. The concentration thus was 10 mM per liter. A stopwatch was started at the time the two pipettes were one-half delivered. The solution was rapidly agitated with a stream of water-washed nitrogen. The pH of the reaction mixture was 7.25.

Samples, 5 ml., of the reaction mixture were removed at intervals up to 24 hours and added to tubes containing 2 ml. of N hydrochloric acid and 25 ml. of water. These tubes were kept in an ice-bath and both the acid and the cold acted to stop the reaction. These samples were then made up to 50 ml. with water and aliquots were immediately taken for measurements of color, fluorescence, and reductic acid. The results are shown in Table 2.

Color was determined by means of an Evelyn photoelectric colorimeter using both the 375 and 400 mμ Evelyn filters. Results are expressed in terms of optical density.

Fluorescence was determined on the Friedemann-Liebeck Fluorimeter, and results are expressed in terms of the equivalent fluorescence of quinine sulfate.

Reductic acid was determined by titration with N/200 iodine and several of the samples were read on the Beckman DU Spectrophotometer as an additional check.

The reactions observed are apparently very complex. The results demonstrate marked increases—but not necessarily parallel—in properties of color, fluorescence, and reducing properties. Color increased rapidly for 2.5 hours and then slowly tapered off. In fact, the 24-hour reading showed very little change from the 5-hour reading.

The fluorescence behaved similarly, with a marked increase for 2 hours and then an actual decrease. The sample at the end of 24 hours had a fluorescence equivalent to that found at 30 minutes.

TABLE 2

Changes occurring in solution of dehydroreductic acid at pH 7.25 and 25° C.

Time of reaction minutes	Reductic acid by iodine mM	Color Optical density		Fluorescence as quinine sulfate mg.
		375 mμ	400 mμ	
1	0	0	0	0.021
2	0	0.02	0	0.032
3	0.04	0.10	0	0.032
4	0.11	0.17	0.04	0.032
5	0.12	0.33	0.15	0.056
7	0.23	0.54	0.30	0.115
10	0.39	1.00	0.50	0.291
15	0.62	1.76	0.96	1.439
20	0.92	2.72	1.55	2.525
30	1.45	4.56	2.58	5.727
45	2.09	6.63	3.86	9.464
60	2.52	8.19	4.79	11.792
90	3.15	8.78	4.90	12.700
120	3.48	9.50	5.40	11.590
150	3.76	10.00	5.70	10.580
180	3.77	10.09	5.70	9.696
210	3.81	10.75	6.03	9.191
240	3.85	10.75	6.09	8.812
300	3.96	10.47	6.04	8.055
24 hrs.	3.62	10.30	6.86	5.828

The reducing activity of the reaction mixture likewise demonstrated a marked increase. Spectrophotometric examination of several of the samples, at 1, 15, 45, and 150 minutes, revealed typical absorption curves of reductic acid with a peak of maximum absorption at 263 mμ. Values for the reducing activity are therefore reported in terms of reductic acid. The pH of the browned solution at the end of 24 hours was 6.98. This represents a decrease from the original pH, 7.25.

The preceding experiment was repeated in the presence of L-tryptophan to determine the effect of this amino acid on the course of the reaction. The procedure followed was exactly that described above except that the reaction mixture contained L-tryptophan in a concentration equimolecular to that of reductic acid, 10 mM per liter. The solution of the amino acid was first neutralized with a determined amount of standard alkali so that the pH of the final mixture was exactly equal to that of the previous experiment, 7.25. Similar determinations of color, fluorescence, and reductic acid were made and the results are shown in Table 3.

Again marked increases in color, fluorescence and reducing properties were observed. The rate of formation and intensity of the color were almost exactly the same as that in the previous experiment. The fluorescence was somewhat higher for the first 30 minutes, due possibly to the tryptophan itself, but after this time the results were very much like those of the previous experiment. The results for the formation of reductic acid were almost identical to those obtained previously. The pH of the reaction mixture after 24 hours was again identical to that of the previous experiment, 6.98.

During the preliminary phases of this work, it was observed that solutions of dehydroascorbic acid underwent a type of browning very similar

TABLE 3

Changes occurring in solution of dehydroreductic acid in the presence of tryptophan at pH 7.25 and 25° C.

Time of reaction minutes	Reductic acid by iodine mM	Color Optical density		Fluorescence as quinine sulfate mg.
		375 mμ	400 mμ	
2	0	0	0	0.028
5	0.18	0.28	0.13	0.137
7	0.23	0.52	0.26	0.235
10	0.41	0.98	0.51	0.416
15	0.68	1.82	0.98	0.822
20	1.01	2.86	1.63	3.323
30	1.58	4.78	2.72	6.424
40	2.12	5.48	2.89	9.050
50	2.56	6.18	3.09	10.933
60	2.87	6.91	3.64	12.070
90	3.55	8.78	4.85	12.398
120	3.88	9.11	4.91	11.767
150	4.03	9.53	5.40	10.555
180	4.05	10.04	5.54	10.050
210	4.08	10.38	5.86	9.166

to that of dehydroreductic acid. The following experiment was set up to investigate this phenomenon under carefully controlled conditions identical to those used for the study of the browning of dehydroreductic acid solutions, i.e., at pH 7.25 and 25° C.

Five mM. of ascorbic acid were treated exactly as was the reductic acid in the experiment on the browning of dehydroreductic acid. Similar observations on the development of color, fluorescence, and reducing substances were made. The initial pH of the reaction mixture was again 7.25.

Results of this experiment are summarized in Table 4. It can be seen that again there resulted an increase in color, fluorescence and reducing properties as occurred with the dehydroreductic acid. By comparison with the dehydroreductic acid reaction, the present reaction occurs much more slowly than does the former. In fact, at the end of 24 hours there appeared only about one-third as much reducing material. In the present experiment, reducing substances are assumed to be ascorbic acid by analogy to the reaction of the dehydroreductic acid. Positive identification of the ascorbic acid was not possible with the techniques as employed for the reductic acid.

In regard to color formation, again much less was developed in the present experiment as compared to the color formed with the dehydroreductic acid. At the end of 24 hours, the optical density of the dehydroreductic acid solution was approximately 13 times that of the present experiment.

Fluorescence similarly was less than that of the dehydroreductic acid, approximately one-thirtieth, and, quite unlike that of dehydroreductic acid, did not reach a maximum value in 1.5 hours. Instead, fluorescence was still increasing when measured at the end of 96 hours.

The pH of the browned dehydroascorbic acid solution was 6.85.

The preceding experiment on the browning of dehydroascorbic acid was repeated in the presence of L-tryptophan to determine the effect of

TABLE 4

Changes occurring in solution of dehydroascorbic acid at pH 7.25 and 25° C.

Time of reaction minutes	Reducing substances as ascorbic acid mM	Color Optical density		Fluorescence as quinine sulfate mg.
		375 mμ	400 mμ	
1	0	0	0.04	0
2	0	0.07	0.07	0.01
10	0.03	0.08	0.09	0.01
20	0.04	0.09	0.11	0.01
40	0.14	0.10	0.12	0.01
Hours				
1.0	0.25	0.12	0.14	0.03
1.5	0.33	0.18	0.15	0.03
2.0	0.45	0.25	0.17	0.05
2.5	0.51	0.29	0.19	0.06
3.0	0.63	0.39	0.23	0.06
3.5	0.71	0.40	0.26	0.07
4.0	0.79	0.48	0.35	0.06
4.5	0.85	0.42	0.20	0.07
5.0	0.89	0.41	0.17	0.06
5.5	0.98	0.51	0.25	0.07
6.0	0.99	0.51	0.26	0.07
24.0	1.10	0.81	0.42	0.19
48.0	1.12	1.33	0.87	0.30
72.0	0.93	1.54	1.11	0.45
96.0	0.86	1.61	1.13	0.56

the amino acid on the course of the reaction. Again, the conditions employed were identical to those of the previous experiments in the series. The concentration of the L-tryptophan was the same as that previously used, 10mM per liter, and the pH of the reaction mixture was again 7.25.

The same observations on color, fluorescence, and reducing properties were made. These results are summarized in Table 5.

The results again indicate increases in color, fluorescence, and reducing properties similar to those obtained with the dehydroascorbic acid alone. No significant effects on rate or intensity of the products were noted after the addition of the tryptophan. In other words, the effect we are seeing is due to the dehydroascorbic acid alone, as in the case of dehydroreductic acid, and not to a Maillard-type reaction.

DISCUSSION

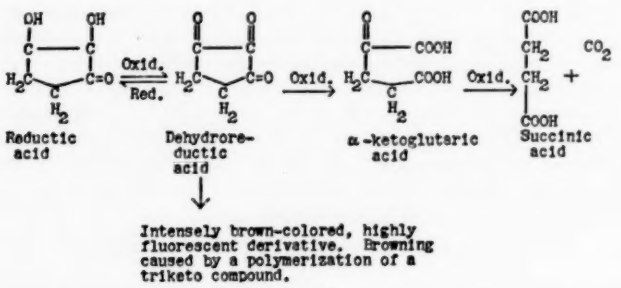
It has been demonstrated that both reductic and ascorbic acids in acid or buffered solutions undergo a type of browning reaction analogous to that occurring in stored or heat-processed foods. The reaction is characterized by an increase in reducing properties, color, acidity, and fluorescence. It was also demonstrated that, in both cases, oxidation of reductic and ascorbic acids to the respective dehydro compound is a prerequisite to browning. In the case of the dehydroreductic acid, the reducing substances formed were identified as reductic acid by spectrophotometric analysis. It is suggested that as dehydroreductic acid is reduced to reductic acid, a simultaneous oxidation occurs which is accompanied by the formation of brown resins, acidic substances, among these probably α-ketoglutaric acid, and other products so far not identified. The following scheme is

TABLE 5

Changes occurring in solution of dehydroascorbic acid in presence of tryptophan at pH 7.25 and 25° C.

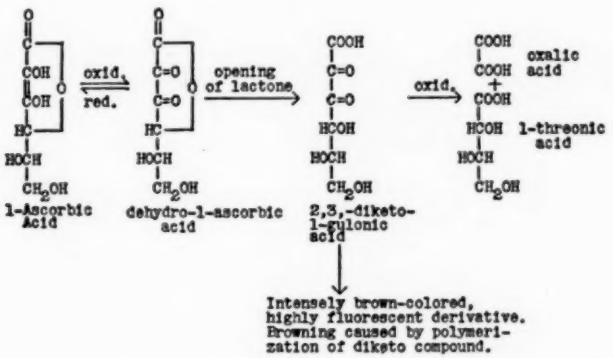
Time of reaction minutes	Reducing substances as ascorbic acid mM	Color Optical density		Fluorescence as quinine sulfate mg.
		375 mμ	400 mμ	
1	0.01	0	0	0
2	0.06	0	0	0
10	0.04	0	0	0
20	0.09	0	0	0
40	0.09	0	0	0.01
Hours				
1.0	0.27	0	0	0.01
1.5	0.33	0	0	0.03
2.0	0.40	0	0	0.04
2.5	0.52	0.08	0	0.07
3.0	0.59	0.17	0	0.08
3.5	0.66	0.35	0.14	0.08
4.0	0.69	0.38	0.14	0.09
4.5	0.71	0.36	0.16	0.10
5.0	0.75	0.40	0.18	0.10
5.5	0.77	0.46	0.22	0.11
6.0	0.81	0.58	0.31	0.12
24.0	1.04	0.92	0.52	0.13
48.0	1.03	1.13	0.56	0.40
120.0	0.88	1.92	1.32	0.82

proposed as an explanation of the reactions which occur during the browning of dehydroreductic acid:



A scheme is also suggested for the browning of dehydroascorbic acid solutions which is similar to that of dehydroreductic acid, except that the reducing substances were not positively identified as ascorbic acid. The degree and rate of browning in a solution of dehydroascorbic acid is very much less than that occurring in a corresponding solution of dehydroreductic acid. This may be due to the fact that dehydroascorbic acid must first undergo an irreversible transformation to 2,3-diketogulonic acid. This transformation was first postulated by Herbert *et al. (1)* and was substantiated by the work of Penney and Zilva *(3)* who studied this reaction under conditions almost identical to those of this investigation. The latter investigators referred to the reaction as a mutarotation. On the basis of

the present information, the accompanying scheme for the browning of dehydroascorbic acid is proposed:



The rate or intensity of the browning of either dehydroreductic or dehydroascorbic acids is not increased by the addition of an amino acid. Thus, the browning is not identical to the Maillard-type of reaction.

It is suggested that the type of browning described above may be one of several mechanisms involved in the browning of foods, especially fruits, since these foods are high in ascorbic acid and may contain reductic acid derived from pectin. Actually, more than one mechanism may be involved at the same time; however, understanding the types of reactions involved may lead to a better means of control with a resultant increase in the acceptability and/or nutritional quality of foods, and to an actual savings in money as a result of decreasing the losses due to the browning reaction.

CONCLUSIONS

It was determined that both reductic and ascorbic acids undergo a type of browning reaction analogous to that occurring in heat-processed and stored foods, i.e., accompanied by marked increases in color, fluorescence, reducing properties, and acidity. Oxidation of these compounds to the dehydro form is a prerequisite to browning and is characterized by simultaneous oxidation-reduction reactions.

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THE EFFECT OF VARIOUS DRYING METHODS ON THE NITROGEN AND FAT CONTENTS OF BIOLOGICAL MATERIALS[a]

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(Manuscript received April 18, 1956)

It is frequently necessary to postpone chemical analyses of biological materials for long periods of time. Many such materials contain a large amount of water and dehydration is desirable from the point of space economy, especially when large amounts of the materials are to be stored. Often, biological materials are more conveniently handled and accurately sampled in the dry state, since they can be pulverized and thoroughly mixed. In our laboratory, we were concerned with determining the nitrogen, fat, calories, and amino acid content of certain foods and of human feces. It was not desirable to add acid to homogenates of this material because caloric and tryptophan values were among the analyses to be done.

The present study was undertaken to show (a) whether or not there is an appreciable loss of nitrogen and volatile fatty acids as a result of drying at the natural pH of biological materials, and (b) to compare common methods of drying for differences in the effect of desiccation on nitrogen and fat contents of such materials. Three foods were selected for carrying out these experiments—ground beef, homogenized cow's milk, and whole eggs. Each of these foods was dried in four ways—air oven, vacuum oven, infrared lamps, and lyophilizer.

EXPERIMENTAL

Preparation of samples for drying. Twenty-four whole eggs were put in a Waring Blendor and homogenized; eighteen approximately 35-g. samples were put in 6-inch uncovered Petri dishes that had been weighed previously. Eighteen 45-g., replicate samples of homogenized milk and eighteen 70-g. samples of ground beef were put in dishes for drying. Samples of similar corresponding sizes were put into weighed flasks to be lyophilized. The weight of food plus container was determined in each case.

Drying procedures. Six samples of each kind of food were put in the air oven at a temperature of 80° C., in the vacuum oven at 61° C., and in the path of infrared rays at an average temperature of 51° C. Three samples of each kind of food were lyophilized at −75° C. and 150 microns pressure. All samples were dried until two successive weighings agreed within 2 mg. The dried samples were ground, transferred to bottles, and stored at −29° C. until analyzed. All analyses were completed within 3 months after drying.

Chemical analyses. Total nitrogen was determined by the Kjeldahl method *(1)*. Packets of Kel-Pak Powder No. 1, consisting of K_2SO_4, $CuSO_4$, and HgO, were used

[a] This investigation was supported in part by funds received from the R. L. Smith Foundation.

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TABLE 1

Per cent moisture, protein, and fat in three foods before and after drying by four methods

Sample	Moisture			Protein[1]			Total fat[1]		
	Egg	Beef	Milk	Egg[2]	Beef[2]	Milk[3]	Egg	Beef	Milk
	%	%	%	%	%	%	%	%	%
Fresh				12.07	23.54 21.54[4]	3.50 3.71[4]	10.43	6.93 11.54[4]	3.76 3.76[4]
Air oven-dried:									
1	72.4	69.2	87.0	12.50	23.78	3.32	11.80	7.39	4.14
2	72.9	70.1	86.9	12.29	23.37	3.35	12.15	6.11	3.20
3	76.1	70.0	86.9	11.55	23.45	3.36	9.69	5.87	4.17
4	74.8	68.0	86.9	11.75	23.69	3.51	10.42	9.00	4.25
5	73.7	68.0	86.9	12.39	22.49	3.24	11.18	8.44	4.30
6	72.9	63.2	86.8	12.08	22.79	3.43	11.08	12.47	4.44
Average	73.8	68.1	86.9	12.09	23.26	3.37	11.05	8.21	4.08
Vacuum oven-dried:									
1	71.4	66.7	86.9	11.88	19.07	3.21	12.34	9.56	4.38
2	72.7	67.2	86.9	12.08	24.60	3.42	11.65	8.86	4.22
3	76.0	66.3	86.9	11.07	22.73	3.44	9.85	8.97	4.37
4	74.3	69.7	86.9	12.42	23.55	3.27	10.54	4.56	4.36
5	72.5	68.4	86.9	12.26	21.77	3.51	11.55	8.77	4.19
6	72.5	68.0	87.0	12.44	22.17	2.81	11.44	8.27	4.23
Average	73.2	67.7	86.9	12.02	22.32	3.28	11.23	8.17	4.29

TABLE 1 (concluded)

Per cent moisture, protein, and fat in three foods before and after drying by four methods

Sample	Moisture			Protein[1]			Total fat[1]		
	Egg	Beef	Milk	Egg[2]	Beef[2]	Milk[3]	Egg	Beef	Milk
	%	%	%	%	%	%	%	%	%
Infrared-dried:									
1	73.6	65.0	86.9	11.99	21.83	3.31	10.36	12.01	4.29
2	74.2	65.4	86.7	11.48	22.49	3.16	10.30	11.35	4.71
3	74.3	66.4	88.7	12.05	22.94	2.90	10.45	10.17	3.75
4	73.6	66.5	87.2	12.18	21.89	3.56	10.57	12.05	4.12
5	73.7	67.6	87.3	12.18	20.92	3.40	10.66	9.70	4.05
6	73.7	67.4	87.2	12.22	22.49	3.47	10.24	9.12	4.27
Average	73.9	66.4	87.3	12.02	22.09	3.30	10.43	10.73	4.20
Lyophilized:									
1	73.8	60.0[4]	85.3	12.42	25.96[4]	3.80	11.15	10.38[4]	4.20
2	74.0	57.3[4]	86.1	12.30	29.02[4]	3.67	10.62	14.12[4]	3.95
3	73.4	61.5[4]	86.8[4]	12.39	22.76[4]	2.80[4]	11.84	10.00[4]	4.06[4]
Average	73.8	59.6	86.0	12.37	25.91	3.42	11.20	11.50	4.07

[1] As determined by analyzing the dried food and using "per cent moisture" in each case for converting to fresh weight basis, i.e., egg sample No. 1, air oven-dried, contained 72.4% water; on analysis for total nitrogen, the dried sample contained 7.25% nitrogen:

$$\% \text{ protein on fresh weight basis} = 7.25 \times \frac{(100.0 - 72.4)}{100} = 12.50$$

[2] Total N × 6.25.

[3] Total N × 6.38.

[4] These analyses were done on a second lot of the food.

in the digestions. Ammonia was distilled into a boric acid mixture. Total fat determinations were made by the Mojonnier method, as modified by Calloway and reported as Method CQD 11 M *(4)*. Analyses on each dried sample were made in duplicate.

RESULTS AND DISCUSSION

Approximately the same amounts of water were removed from whole egg and ground beef by all 4 methods of drying. Averages for duplicate determinations are presented in Table 1. In the case of ground beef, a second lot of beef had to be used for freeze-drying, and the amount of water removed, 59.6%, is not directly comparable with the amounts of water removed in the air oven, vacuum oven, and by infrared radiation, namely, 68.1%, 67.7%, and 66.4%, respectively. The third sample of milk for lyophilization also had to be obtained from a second lot. Analysis of variance of moisture data for the 3 foods showed that differences in the amounts of water removed from egg and beef by the 4 methods were insignificant (lyophilized beef was omitted in the analysis); differences in the amounts of water removed from milk by the 4 methods were significant at the 1% level.

Protein and fat contents of fresh and dried samples of the 3 foods are reported in Table 1. Mean per cent of nitrogen and of fat retained in these foods after drying is reported in Table 2. Numerically, the per cent retention of nitrogen in the 3 foods was highest in lyophilized samples. Next highest retention of N in all cases was in air oven-dried foods. Statistically, the differences in N retention of the 3 foods dried in 4 ways were insignificant at the 1% level, and significant at the 5% level only in the case of beef.

Several investigators have reported N losses in biological materials that were not acidified before desiccating *(2, 3, 5)*. Teague *et al.* reported that drying fecal material in the oven caused an 18% loss in N content of an acid digest of fresh feces *(6)*. The pH of the fresh foods in this study was determined before drying:

Homogenized milk	pH 7.2
Ground beef	pH 7.7
Whole egg	pH 7.9

High N retentions in these experiments cannot be associated with natural acidity of the foods.

Numerically, the per cent total fat retained in beef and milk after lyophilization was lower than in these foods dried by the other 3 methods (Table 2). All mean fat retentions, except in the case of lyophilized beef, were greater than 100%. These results may be due to (a) oxidation, which is minimal in freeze-drying because of low oxygen tension and absence of heat, and (b) the fact that drying eliminates the aqueous phase and thereby increases the total amount of material extracted in the ether phase. Largest increase in fat on drying occurred in infrared-dried ground beef; this set of dried samples contained an average of 54.9% more fat than did the fresh samples. Differences in fat retention for the 3 foods dried by 4 methods were all insignificant at the 1% level. At the 5% level, fat retentions in ground beef dried by 4 methods differed significantly (Table 2).

TABLE 2

Mean per cent nitrogen and fat recovered from foods after drying by various methods

Biological material	Total nitrogen					Total fat				
	Air oven-dried	Vacuum oven-dried	Infrared-dried	Lyophilized	Variance ratio	Air oven-dried	Vacuum oven-dried	Infrared-dried	Lyophilized	Variance ratio
	%	%	%	%		%	%	%	%	
Whole egg	100.2	99.6	99.6	102.5	0.68[1]	106.0	107.6	100.0	107.4	1.50[1]
Ground beef	99.8	94.8	93.8	110.1	4.62[1]	118.5	117.8	154.9	99.7	3.72[1]
Homogenized milk	96.2	93.6	94.3	106.7	2.78[2]	108.6	114.1	111.7	108.2	0.61[1]

[1] Significant F (3 and 17 degrees of freedom) = 5.18 at 1% level and 3.20 at 5% level.
[2] Significant F (3 and 16 degrees of freedom) = 5.29 at 1% level and 3.24 at 5% level.

SUMMARY

Statistically, there was no difference at the 1% level in per cent N and fat retained in egg, ground beef, and whole milk on drying by the air oven, vacuum oven, infrared rays, and lyophilizer. Among the 4 methods studied, the authors prefer lyophilization because the physical qualities of the lyophilized material are superior to heat-dried samples, the drying procedure is the least time consuming, and the average fat retention most nearly approximates 100%.

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GROUP SIZE IN TASTE SORTING TRIALS[a]

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(Manuscript received April 28, 1956)

In a celebrated chapter Sir Ronald Fisher *(1)* introduces a lady who avers that she can tell from the taste of a cup of tea whether, in the making, the infusion or the milk has been poured first. Then, somewhat ungallantly, he proceeds to design a suitable lie-detector in the form of an experiment with 8 cups of tea to put the alleged faculty to direct test. Sir Ronald proposes that the lady be confronted with these cups, 4 prepared one way and 4 the other, in coded random order, and that she be asked to sort them, by taste, into their two proper groups. It may be shown that, if her decisions are pure guesswork, the odds against her turning in an all-correct solution are 69 to 1. If, therefore, her actual solution *is* faultless, the inference may fairly be made that she is not guessing but exercising true discrimination. On the other hand, a single mistake necessitates the verdict: Not proven.

Fisher, of course, uses this illustration merely as a peg on which to hang a concinnous exposition of the logic of the laboratory. In fact it is but one of a family of designs available for the purpose [see, also, Wood *(4)* and Lockhart *(3)*], which may be generalized as the sensory detection of small constitutional differences between two materials. More particularly, consider a suspected marginal flavor difference between two ostensibly identical foods, and how best to present samples of them to a taste panel charged with its detection. This is an important food technology counterpart to the hypothetical tea experiment. The family of designs consists of pairs, tetrads, hexads, octads, and so on, and the question may be posed: Do they differ in efficiency? Is a subject more or less likely to detect a difference when he has to sort a block of 8 samples than when he is given 4 separate pair comparisons? And what of the intermediate design, namely, the sorting, separately, of two blocks of 4 samples? Here are three arrangements, all involving the same number of samples to be dichotomously sorted by taste, on whose relative merits it would be useful to have information. This paper presents some.

Theoretical considerations. First thoughts on the subject are likely to isolate two points of interest. One is that the larger the unit the more psychophysically complex is the taster's task, and this might be expected to redound to the disadvantage of a big unit such as an octad in competition with multiple smaller units such as pairs. The other point is that the larger the unit the smaller will be the probability of a pure-chance selection of 4 specified items. The significance of this fact is however easily misunderstood, and we may profitably look further into it, especially as it can lead us some way to the heart of the matter, which is how efficiency is to be assessed.

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[a] Issued as N.R.C. No. 4102.

Our 8 cups of tea will conveniently illustrate the underpinning theory. Let us denote one of the two types of preparation by A, B, C, D and the other by a, b, c, d. If they are served to the taster as 4 separate comparisons, A:a, B:b, C:c, D:d, the number of possible selections for either group (say, the presumed "milk first") is 16, made up as follows: ABCD, ABCd, ABcD, AbCD, aBCD, ABcd, AbCd, aBCd, AbcD, aBcD, abCD, Abcd, aBcd, abCd, abcD, abcd. Supposing the capital letters to represent the true "milk first" cups, we see that there is 1 selection with no errors, 4 with one, 6 with two, 4 with three, and 1 with all four items in error. But if the cups are served as duplicate tetrads, A:B:a:b and C:D:c:d, the numbers of possible selections with one, two, or three errors will be *ipso facto* increased. For example, the single-error selections will be doubled by the addition of ABCc, ABdD, AaCD, and bBCD; and the augmentations of the other subgroups are not difficult to work out. Similarly, if the servings form a single octad, still further increases in the selection possibilities can be demonstrated. In sum, the results are collected in

TABLE 1

Possible selections in dichotomous sorting of 8 items

Number (in one sorted group of 4) having an attribute characteristic of half the items.	Limiting relative frequency of the different selections by chance alone when the items form:		
	4 pairs	2 tetrads	1 octad
4	1	1	1
3	4	8	16
2	6	18	36
1	4	8	16
0	1	1	1
	16	36	70

N.B. The pairs, and the tetrads, are sorted independently.

Table 1, and from them we infer that a non-discriminating taster could turn in an all-correct solution by lucky guess with a frequency of 1 in 16 for a set of 4 pairs, of 1 in 36 for a set of 2 tetrads, and of 1 in 70 for an octad. Inversion of this argument allows us to say that the larger the unit the greater the statistical significance of an observed all-correct solution. Now this fact is interesting and pertinent, yet it gives no direct clue to the answer to the inquiry, for we have to consider the frequency of error-free and near error-free selection *when a modicum of taste discrimination exists.* Specifically, we need the probability of a taster's correctly rejecting the no-difference hypothesis when a difference is actually but unobviously present. Estimated for the three methods of sample presentation, this probability will measure their relative *power* for any common frequency of correct judgements.

But this theoretical approach takes us only part way to a solution of the problem, for in practice it is hardly to be expected that the several methods will give rise to identical frequencies of correct results. This suggests an experimental approach. There is no difficulty about specifying significance tests for pair-comparison and sorting results. If, then, we present a subject with the same flavor contrast, firstly as N octads, and

secondly as 4N pairs, the more powerful method will show the greater incidence of statistically significant results. There are theoretical reasons for supposing that the power of the method of tetrad sorting will fall somewhere between the other two.

EXPERIMENTAL

Two qualitatively different flavor contrasts were used. One was generated by the following mixture (parts by weight) of primary flavors: 1 quinine sulfate, 175 tartaric acid, 500 sodium chloride, 3125 sucrose; and the actual contrast was provided by aqueous solutions at 0.51% (labelled F_1) and at 0.57% (labelled F_2). The other contrast was between regular canned tomato juice (J_1) and the same material plus 0.1% salt (J_2). Both the contrasts were settled on by a process of trial and error, for it had to be arranged that they fell somewhere between the extremes of undetectableness and obviousness.

A panel of 6 subjects did the tasting over a series of 6 mid-morning or mid-afternoon sessions carried out in individual air-conditioned booths. At any one session a subject was confronted with either (i) a (coded) octad of primary flavors (4 each of F_1 and F_2), followed by 4 coded pairs of the tomato juices J_1 and J_2, or (ii) ditto, in reverse order, or (iii) an octad of J_1 and J_2 followed by pairs of F_1 and F_2, or (iv) ditto, in reverse order. These alternatives were randomized over all 6×6 subject-sessions, with the restriction that each was equally represented. The reference letters on the samples were always identical but the key differed for each session. It was so arranged that any possible bias due to order of tasting or of presentation was compensated; and here again the orders were randomized within that restriction. The subjects were asked to select the stronger flavored or the saltier, as the case might be, and to record their decisions on a questionnaire. Always, a decision had to be made, "can't tell" being disallowed.

RESULTS

Results are assembled in Table 2, as numbers of correct decision in 24, by subjects, designs, and materials. It is demonstrable (see next section) that at least 17-in-24 correct decisions in pair comparisons have to be made before we can accept the existence of a flavor difference at the $P = 0.05$ level. Similarly, at least 16-in-24 correct in octad sorting are necessary for the acceptance. In the present experiments 7 of the "pair" results and 3 of the "octad" results are at or beyond these acceptance levels. To this extent we infer that pair comparisons have been more successful than octad sorting in the detection of the imposed differences.

DISCUSSION

A probabilistic model for sensory pair comparison can be constructed with reasonable confidence. We envisage a probability of discrimination p_1 and combine it with the conditional probability of correct judgement

TABLE 2

Observed frequencies, in a possible 24, of correct placements in a taste-sorting experiment

Contrast	Design	Subject reference:						
		S_1	S_2	S_3	S_4	S_5	S_6	Total
Mixed flavor	24 pairs	15	18*	19*	16	12	18*	98
	6 octads	14	12	16*	17*	13	15	87
Tomato juices	24 pairs	10	17*	23*	19*	13	15	97
	6 octads	14	13	16*	13	14	12	82

N.B. Asterisked values are significantly greater than the null hypothesis expectation of $\bar{X}/N = 12$.

by chance—which is ½. Thus the observed probability of selection of a specified member of the coded pair is $(1 + p_1)/2$. But the mechanism of the sorting procedure is more complex and a satisfactory model is less easily framed. A simple empirical course is to let the same p_1 apply to the sensory detection of a single item in the dichotomous sorting (for the absence or presence of a given attribute) from a group of 2N. (This is not to say that, *ceteris paribus*, p_1 will take the same numerical value for pairs and octads; in fact we may expect it to be smaller for the more complex set-up.) Assume now that the fractional frequency of X, the number of correct items in the sorted-off sub-group of N, is

$$\text{pr}(X) = \frac{\binom{N}{X}^2 (1+p_1)^N \ (1-p_1)^{N-X} \, 2^{X-2N}}{\sum_{R=0}^{N} \binom{N}{R}^2 (1+p_1)^N \ (1-p_1)^{N-R} \, 2^{R-2N}} \qquad (1)$$

which is an adaptation of the binomial distribution with "squared coefficients."

An alternative model starts from the notion of (i) a probability p_2 of the sensory detection of one item; (ii) a binomial distribution of the probability of the sensory detection of X items (X = O to N); and (iii) a conditional probability of the chance addition of further items from the remaining $N - X$ when the sorted sub-group is being made up to the requisite N. In this event it may be shown that

$$\text{pr}(X) = \sum_{R=0}^{N} \frac{\binom{N}{R}\binom{N}{X}\binom{N-R}{N-X}}{\binom{2N-R}{N}} \, p_2^N (1-p_2)^{N-R} \qquad (2)$$

$$= \sum_{R=0}^{N} \frac{(N!)^3 (N-R)!}{R!\,X!\,(2N-R)!\,[(N-X)!]^2\,(X-R)!} \, p_2^N (1-p_2)^{N-R}$$

Operationally, the two models are almost identical. This is evident from inspection of Table 3 in which the results, as frequencies, for the 6 subjects are summed—the inter-subject differences are not statistically significant. Both also satisfy certain important requirements. When p_1 or p_2 is zero, the sorting is purely guesswork and the two expressions each produce the "null" values. (For instance, with N = 4, they give 1/70 for X = 0 or 4; and 16/70 for X = 1 or 3; and 36/70 for X = 2.) In that event the expectation of $\bar{X}$ becomes N/2. When N = 2, the pair comparison situation obtains and p_1 and p_2 become identical. For M replicate groups, separately sorted but whose results are pooled, the possible values of X will range from MN to zero, and their limiting frequencies can be calculated from the summed diagonals (MN + 1 in number) of an M-dimensional multiplication matrix whose margins are the N + 1 values of expressions (1) or

TABLE 3

Frequencies of correct judgement (6 subjects' results pooled)

Material	Number correct in 4	As 4 pairs		As 1 octad		
		Observed	Expected (binomial)	Observed	Expected	
					(Model 1)	(Model 2)
Mixed flavors	4	9	7.70	4	1.10	2.20
	3	13	14.49	12	11.78	14.31
	2	10	10.23	15	17.67	15.88
	1	3	3.21	5	5.23	3.67
	0	1	0.38	0	0.22	0.12
	χ^2		0.42		1.20	0.44
	df		3		2	2
Tomato juice	4	10	7.25	2	0.88	1.40
	3	11	14.29	13	10.66	12.66
	2	10	10.56	14	18.10	17.06
	1	4	3.47	7	6.07	4.69
	0	1	0.43	0	0.29	0.19
	χ^2		2.08		2.04	1.53
	df		3		2	2

(2). This enables the double-tetrad situation to be compared to the octad situation (and, of course, multiples of them, as in the present experiments).

Table 4 gives some theoretical probabilities of interest in connection with the power concept. The columns headed "expected $\overline{X}/N = 0.5$" contain the probabilities of certain values of X (ranging from 1 to 24) for 24 pairs or 6 octads when discrimination is absent. It is from these data that the rule stems: At the 5% acceptance level X must be $\geqslant 17$ for pairs, or $\geqslant 16$ for octads. Suppose now that a certain subject has a limiting value of $\overline{X}/N = 0.59$ for both octads and pairs with a particular flavor contrast. We read from the table that the "power" probability for pairs would, at 0.1660, be rather less than the corresponding value of 0.2222 for octads. But if his limiting value for $\overline{X}/N$ happened to be as high as

TABLE 4

Probabilities that dichotomous sorting of 48 samples will congregate X or more correct items in 24

X	Expected $\overline{X}/N$ with 24 pairs:—			Expected $\overline{X}/N$ with 6 octads:—		
	0.5	0.59	0.68	0.5	0.59	0.59
					Model 1	*Model 2*
24	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
18	0.0113	0.0803	0.3103	0.0012	0.0291	0.0310
17	0.0320	0.1660	0.4794	0.0067	0.0925	0.0956
16	0.0758	0.2926	0.6484	0.0277	0.2222	0.2250
15	0.1537	0.4490	0.7898	0.0862	0.4169	0.4170
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
1	0.9999+	0.9999+	0.9999+	0.9999+	0.9999+	0.9999+

0.68 for pairs, the "power" probability for this design would, at 0.4794, be appreciably the greater. All this applies specifically to the 5% acceptance level, of course. The particular values for $\overline{X}/N$ are chosen because of their being the estimates in the present trials (whose *limiting* values, however, we do not know, and it would require a very big trial to ascertain them with precision).

Similar calculations and experiments could also be made for tetrads, hexads, etc., as well as for imbalanced groups [e.g., the 2-in-5 method discussed by Harries *(2)*]. However, if the present findings make a reliable guide, the first hypothesis would be that complex designs in general have no advantages over the method of pair comparison. Simplicity and efficiency seem to be strongly correlated in this field.

SUMMARY

Experimental trials indicate that small flavor differences are more efficiently detected when the coded samples are presented as 4N pair comparisons than as N octads to be sorted by taste. The statistical theory of such trials is discussed. The tentative generalization is made that the larger the group presented to the subject in a taste trial the less efficiently can he exercise his ability to discriminate.

Acknowledgements

I am glad to be able to thank Dr. J. W. Hopkins for the suggestions that lead to formula 2, and Mrs. Gladys Tyler for the organization and conduct of the trials.

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VITAMIN B_{12} POTENCY OF CHEDDAR, SWISS, AND COTTAGE CHEESE

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(Manuscript received April 28, 1956)

Not many figures for the vitamin B_{12} potency of various cheeses have appeared in the literature. By use of an assay method utilizing the hyperthyroid rat, Lewis *et al. (7)* found a sample of Cheddar cheese to contain 14 mcg. vitamin B_{12} per kg. (23 mcg. per kg. of dry matter). Elvehjem *(1)* reported a value of 25 mcg. per kg. of dry matter for this type of cheese by *L. leichmannii* assay. Hietaranta and Antila *(4)* found two samples of Swiss (Emmental) cheese, assayed microbiologically with *L. leichmannii*, to average 25 mcg. per kg. after 3 months of ripening and two samples to average 32 mcg. per kg. after 5.5 months of ripening.

The present paper contains the results of assays of a number of samples of Cheddar, Swiss, and cottage cheese. The vitamin B_{12} content of these samples was determined both by a microbiological and by a rat assay method.

EXPERIMENTAL

In order to obtain information on any possible changes that might occur in the vitamin B_{12} potency of Cheddar cheese during the curing process, a single lot of curd was prepared[a] from one lot of pasteurized milk. The curd was divided and pressed into 10 pound blocks which were wrapped in "Parakote." A portion of one block was taken for immediate assay. Three blocks were placed in a room at about 55° F. for assay after 2, 4, and 6 months. Four blocks were placed in a room at about 40° F. for assay after 6, 9, 12, and 18 months. At the intervals indicated, a block was removed from the curing room for assay and treated in the same manner as described below for the commercial samples of cheese.

With the exception of the above lot of Cheddar and one lot of natural Swiss cheese,[a] samples were purchased from 4 retail groceries and one dairy in the Washington, D. C. metropolitan area. Where different samples of the same brand were assayed, they were purchased at intervals of one to two months apart. Each sample of Cheddar or Swiss cheese was subdivided into small portions, placed in tightly closed containers, and stored in a deep freeze at about −18° C. to obviate or at least minimize any bacterial or enzyme action during the assay period. With each sample of cottage cheese, the contents of several cartons were pooled, mixed with a mechanical food mixer, and then similarly subdivided and stored. For rat assays, a cheese container was removed from the refrigerator and brought to room temperature each day that portions were fed to the assay animals.

The rat growth assay method used has been described in a previous report *(3)*. In the present work, supplements of cheese and of vitamin B_{12} standard were fed

[a] Prepared by Dairy Products Research Section, Eastern Utilization Research Branch, Beltsville, Maryland.

separately from the ration. Results of the assays have been calculated by use of the combined slope (for the B_{12} standard) which was developed in the above-mentioned report for the growth-log dose regression found to be applicable to separately-fed supplements. The combined slope for natural Cheddar cheese derived from the regression lines in 8 assays did not differ significantly from that obtained for the B_{12} standard; moreover, error variance of the cheese and B_{12} groups of assays were statistically homogeneous. Furthermore, when a supplement of natural Cheddar cheese was fed to assay rats in addition to maximally effective amounts of vitamin B_{12}, growth over the assay period was not different from that obtained when the vitamin B_{12} alone was fed. Thus, this method appeared to be specific for the vitamin B_{12} activity of Cheddar cheese. To secure some indication of the precision obtained in these assays, two determinations were made on each of 5 samples of cheese (1 Cheddar, 3 Swiss and 1 cottage). The two assays were carried out simultaneously in one instance and from one to 6 months apart in the other 4 cases. The average of the second assays of these 5 samples differed from the average of the first assays by about 11%, which is no greater than the experimental error of the method as determined from the data for the B_{12} standard *(3)*.

The U.S.P. microbiological assay procedure *(8)* for vitamin B_{12} activity was followed using *Lactobacillus leichmannii* strain No. 7830 and Difco dehydrated U.S.P. medium.

Frozen cheese samples were brought to room temperature and treated in the following manner for assay: Samples of cheese were shredded and 10 g. aliquots were placed in a semi-micro Waring blender, the volume brought up to 100 ml. with hot (50° C.) aqueous emulsifying solution, and emulsified for 3 minutes. The emulsifying solution was prepared fresh prior to use to contain, in each 100 ml., 1.36 g. of monopotassium phosphate, 0.75 g. of sodium tetraphosphate and 5 mg. of potassium cyanide. One ml. of cheese emulsion was diluted with an appropriate volume of water, usually at the ratio of 1:1000, so that the test solution would have approximately the same activity as the standard cyanocobalamin solution. Since many of the cheese test solutions were slightly turbid, bacterial growth responses in the assay were measured by determining titratable acidities.

The preceding method for treating cheese samples resulted from a comparative study of various extraction and emulsifying procedures. Potassium cyanide and sodium bisulfite were compared as stabilizing agents for B_{12} activity in the presence of heat. No loss in activity was noted with either substance even with autoclaving at 121° C. for 15 minutes. Sodium citrate, sodium tetraphosphate, and detergent X-100[b] were compared as emulsifying agents for cheese. Of these 3 agents, sodium tetraphosphate appeared to be most suitable.

Because of lack of agreement between values obtained with the rat and microbial assay, further attempts were made without success to liberate additional B_{12} activity from the cheese by heat and further chemical treatment. Filtrates of treated samples were measured for B_{12} activity in assay both by acidimetric and photometric procedures. Replicate tests with emulsified cheese preparations showed less deviation in assay values than did the filtrates of cheese although average values from each were approximately the same. Known amounts of standard B_{12} added to the cheese emulsions yielded quantitative responses with the microbial assay.

RESULTS AND DISCUSSION

Values obtained for the vitamin B_{12} potency of the various types of cheese are shown in Table 1.

With natural Cheddar cheese, rat assay indicated an average of 20.8 mcg. vitamin B_{12} per kg. of cheese, whereas microbiological assay indicated only about half this potency. By rat assay, Process Cheddar appeared to have an average potency of only about three-fifths that of natural Cheddar

[b] Rohm and Haas Company, Philadelphia, Pennsylvania.

TABLE 1

Vitamin B_{12} potency of cheese

Type of cheese	No. of samples	No. of brands	Vitamin B_{12} Content			
			Microbiological assay (Fresh basis)	Rat assay (Fresh basis)	Rat assay (Dry basis)	Ratio of microbiological to rat assay value
			mcg./kg.	*mcg./kg.*	*mcg./kg.*	
Natural Cheddar						
Sharp	3	3	9.0	18.3	28.7	0.49
Medium	2	1	10.0	18.0	29.0	0.56
Mild	3	2	11.0	22.3	35.3	0.49
Average	9[1]	5	10.3	20.8	32.8	0.50
Range[2]			7-13	12-29	18-45	0.37-0.83
Process Cheddar						
Sharp	2	2	9.5	14.0	23.0	0.68
Mild	3	3	7.0	10.7	17.0	0.65
Average	5	4	8.0	12.0	19.4	0.67
Range[2]			6-12	9-14	14-24	0.50-0.86
Process Cheddar Cheese Food						
Average	2	2	6.5	8.5	15.5	0.76
Range[2]			6-7	8-9	15-16	0.67-0.88
Process Cheddar Cheese Spread	1	1	4	5	12	0.80
Natural Swiss						
Average	6	4	20.8	36.2	57.5	0.57
Range[2]			19-22	22-54	36-87	0.35-1.00
Process Swiss						
Average	5	4	12.2	18.4	32.0	0.66
Range[3]			8-17	10-29	18-51	0.55-1.10
Cottage						
Large curd	3	2	8.3	7.3	34.0	1.14
Small curd	2	2	9.5[3]	9.0	44.5	1.06
Average	5	3	8.8	8.0	38.2	1.10
Range[2]			7-12	5-10	21-49	0.70-1.80

[1] Includes one lot prepared at Beltsville; the value for this lot is the average of 7 assays carried out 2-18 months after manufacture; see Table 2.
[2] Of individual samples.
[3] An additional sample gave 5 mcg./kg. by microbiological assay but was not measurable by rat assay at a dose which gave positive responses with other samples of cottage cheese.

cheese. On the other hand, as judged by microbiological assay, there was much less, if any real, difference between the B_{12} potencies of these two types of cheese. Process Cheese Food and Process Cheese Spread, both made from Cheddar cheese, were somewhat less potent than Process Cheddar itself, irrespective of which method of assay was used. No correlation between mildness or sharpness of Cheddar cheese and vitamin B_{12} potency was observed.

The vitamin B_{12} potency of natural Swiss cheese was found to average about one and three-fourths to twice that of natural Cheddar. Process Swiss cheese had about 50 to 60% the potency of natural Swiss. Although, with most of the cheeses tested, there seemed to be no consistent variation

with brand, it was observed with Process Swiss cheese that two samples of one brand gave B_{12} values that were about double those of single samples of 3 other brands tested.

With cottage cheese, the microbiological and rat assay methods indicated about the same average potency (8.8 and 8.0 mcg. per kg.). There was no indication of any correlation between curd size and B_{12} potency.

As determined by rat assay, natural Cheddar, Process Swiss, and cottage cheese had on the average about the same potency on a dry matter basis (33, 32, and 38 mcg. per kg.). On the same basis, Process Cheddar had a smaller average potency (19 mcg. per kg.) while natural Swiss had a greater average potency (58 mcg. per kg.) than these 3 cheeses.

The results of the assay of one lot of Cheddar cheese, divided into two parts, each cured at a different temperature, and tested after certain intervals of time had elapsed, are shown in Table 2. Within the limits of the

TABLE 2

Vitamin B_{12} potency of Cheddar cheese after various periods of curing at different temperatures[1]

Curing temperature	Period of curing	Vitamin B_{12} content[2]		
		Microbiological assay (Fresh basis)	Rat assay	
			(Fresh basis)	(Dry basis)
° F.	*months*	*mcg./kg.*	*mcg./kg.*	*mcg./kg.*
....	(Original curd)	12	32	50
55	2	13	26	41
55	4	13	26	41
55	6	12	29	46
55	average	12.7	27.0	42.7
40	6	13	31	48
40	9	14	30	47
40	12	13	38	58
40	18	15	23	36
40	average	13.8	30.5	47.2

[1] Single lot of curd prepared at Beltsville, divided into two parts, each cured at a given temperature for various periods.
[2] Ratio of microbiological to rat assay value: 0.45 (0.34-0.65).

precision of the assay methods used, there did not appear to be any change in the vitamin B_{12} potency during curing at either 55° F. or 40° F., nor did curing temperature affect the B_{12} potency. These temperatures approximate those commonly employed in commercial practice.

With most but not all types of cheese, the rat assay values obtained were generally higher than the microbiological assay values. Thus, with natural Cheddar cheese, the rat method gave higher results with every sample, the microbiological values averaging only 50% as much. With Process Cheddar, Process Cheese Food, and Process Cheese Spread, the rat method likewise gave higher values in all instances, but the differences between the results obtained by the two methods were not as great as with natural Cheddar, the microbiological values averaging 70% of the rat

values. For natural Swiss and Process Swiss cheese, the microbiological values averaged, respectively, 57% and 66% of the rat assay values. On the other hand, with cottage cheese, no difference was found on the average between the potencies determined by the two methods of assay.

An explanation for the divergent results obtained with the two assay methods is not readily apparent. As discussed previously, the rat method appeared to be specific for the vitamin B_{12} activity of cheese, at least of natural Cheddar, where the differences obtained by the two methods were quite pronounced. Thus it seems improbable that the rats may have been responding to some other nutrient besides B_{12} in this milk product. It is conceivable that certain microorganisms present in cheese (particularly Swiss) may have become implanted in the intestinal tract of the rat and promoted intestinal synthesis of vitamin B_{12}. Such synthesis, if it took place, would obviously lead to erroneously high values. However, growth curves of the assay rats gave no indication that such synthesis was taking place. Moreover, it will be observed (Table 2) that, with Cheddar cheese made from milk which had been pasteurized and in which most microorganisms were presumably destroyed, a considerable difference between the rat and microbiological assay values was still observed, both with the original curd and later during curing.

Another possible explanation is that part of the vitamin B_{12} in cheeses other than cottage may have been unavailable to the assay microorganisms although attempts to liberate any additional vitamin B_{12} activity were without success. Still another possibility is that some of the vitamin B_{12} activity of such cheeses was destroyed in preparing the samples for microbiological assay, although cyanide was used in this process.

It is, of course, conceivable that a different assay microorganism might give results in closer agreement with those of the rat assays.

The greater vitamin B_{12} potency of Swiss cheese as compared to the other cheeses is probably due to the synthesis of vitamin B_{12} by propionic acid bacteria *(2, 5, 6)* which are involved in the manufacture of this kind of cheese. Apparently in the preparation of Process Swiss cheese from the natural product, some vitamin B_{12} activity is lost. This conclusion is indicated by both assay methods. If the rat figures are the true ones, such loss also occurs in the preparation of Process Cheddar from natural Cheddar cheese. It is interesting to note that there is some similarity in the treatment of the cheese samples for microbiological assay and in the manufacture of process cheese, in that heating with an emulsifying agent is involved in both procedures. It is conceivable that this parallelism in treatment could afford at least a partial explanation for the differences in the values obtained by the two assay methods, since the samples used for the rat method were not so treated.

SUMMARY

The vitamin B_{12} potency of samples of natural Cheddar, Process Cheddar, Process (Cheddar) Cheese Food, Process (Cheddar) Cheese Spread, natural Swiss, Process Swiss, and cottage cheese were determined by both microbiological and rat assay. Agreement of the results obtained by the two methods was much better with cottage cheese than with the other types of cheese. No correlation was observed between mildness or sharp-

ness of Cheddar cheese and vitamin B_{12} potency or between curd size in cottage cheese and potency. The vitamin B_{12} content of one lot of natural Cheddar cheese did not change during the curing process. On a dry matter basis, natural Cheddar, Process Swiss, and cottage cheese had on the average about the same potency (33, 32, and 38 mcg. per kg., respectively) as determined by rat assay. Process Cheddar was less potent (19 mcg. per kg.), whereas natural Swiss was more potent (58 mcg. per kg.). Possible explanations for differences in potency of different types of cheese and for the differences in the values indicated by the two assay methods have been considered.

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TIME OF LOADING AND STRAIN RETARDATION IN STARCH JELLY CANDY

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(Manuscript received May 11, 1956)

An earlier investigation *(11)* considered the rôle of varied quantities of stress, applied for a constant period of time, on retardation of strain in a starch gel. Although that study proposed a tentative mechanical model to describe some aspects of gel behavior, only a limited representativeness could be ascribed to the model. To judge further the utility of such a model and to gain a more comprehensive picture of the rheological properties of the gel system, it seems desirable to ascertain the effects of altering the time of stress. The present study deals with the rôle of a constant stress, applied for varying periods of time, on retardation of strain.

MATERIALS AND METHODS

The composition of the starch jelly and its method of preparation have been described previously *(11)*. Similarly, the manner of applying stress and the recording of the strain/time curve follow the earlier description *(2)*. A 50 g. load was applied for periods of 1, 5, 10, 30, 60, 300, 600, and 1200 seconds, respectively, to series of candy samples. After each stress application, the gel was allowed to recover for a 20-minute period. A different sample was used for each determination. Since there is relatively little change in stress values after the first few seconds of loading *(11)*, the stress was assumed to be constant throughout the period of creep.

In addition, a further type of experiment was performed. This consisted of loading the gel with 50 g. for a period of time, allowing it to recover for 1200 seconds (20 minutes), loading again with the same load for 1200 seconds, and permitting the gel to recover for a further 1200 seconds. Two time periods of initial loading were used: 60 seconds (I_{60}) and 1200 seconds (I_{1200}). The rationale of this experiment will be developed in the discussion, below.

TABLE 1

Percentage strain during recovery for varying times of application of 50 gm. load

Time of recovery (seconds)	Time of stress (seconds)							
	1	5	10	30	60	300	600	1200
0 { Moment of unloading	13.01	20.19	21.18	21.92	23.01	24.69	24.84	25.62
5	2.02	3.30	3.54	4.72	5.48	8.23	9.66	12.28
10	1.67	2.51	2.93	3.95	4.63	7.32	8.89	11.18
20	1.47	2.02	2.49	3.34	4.00	6.48	7.88	10.08
40	1.36	1.81	2.24	2.94	3.47	5.78	7.05	9.13
60	1.31	1.74	2.12	2.78	3.24	5.41	6.66	8.68
300	1.27	1.67	2.04	2.46	2.73	4.54	5.60	7.45
600	1.25	1.63	1.99	2.38	2.57	4.30	5.33	6.97
1200	1.25	1.62	1.97	2.34	2.49	4.19	5.19	6.72

RESULTS

Table 1 presents the data of amounts of strain after various periods of loading and during the subsequent recovery. If the unrecovered strain, after 20 minutes of recovery, is plotted against the time of application of stress (Figure 1), is is clear

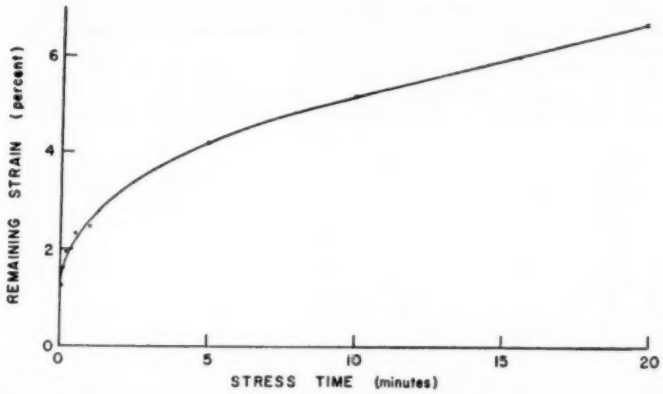

Figure 1. Amount of strain remaining after 20 minutes of recovery vs. time of application of stress.

that the relationship is not rectilinear. The permanent[a] deformation increases very rapidly in the first few seconds, with the rate of increase becoming more and more retarded as the time of application of stress increases.

In Figure 2, amount of strain, for both the total deformation and the unrecovered deformation, is plotted against the logarithm of time of stress application. Although

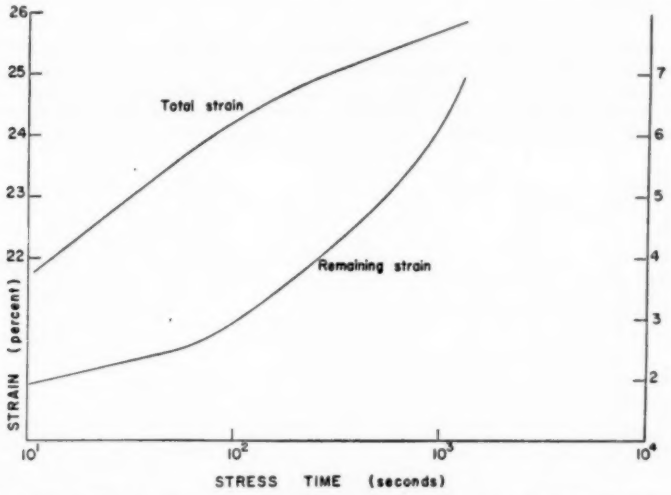

Figure 2. Total strain on loading and strain remaining after 20 minutes of recovery vs. logarithm of time of application of stress. Remaining strain values are to be read from right-hand side of graph.

[a] As in the earlier study *(11)*, the permanent deformation is that amount of strain remaining after 20 minutes of recovery.

the total deformation increases more rapidly in the earlier periods of stress, there is a relatively greater rate of increase in the amount of permanent deformation on a logarithmic scale as the time under stress continues.

If, as defined earlier *(11)*, "resilience" may be taken as the ratio of recovered deformation to the total deformation, the resilience of the starch gel decreases with the time of stress (Figure 3). There is an inverse relationship of this curve to that of

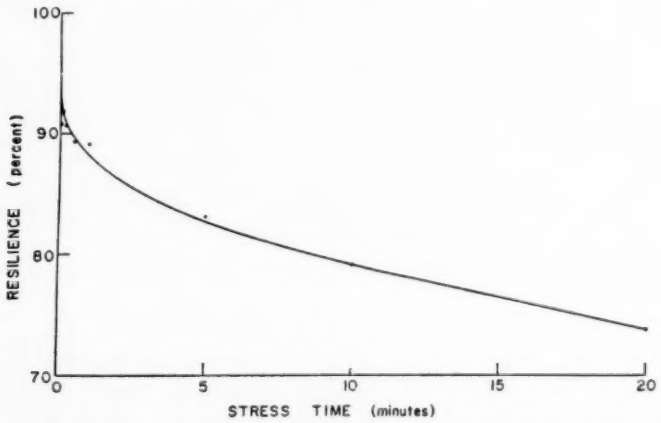

Figure 3. Per cent resilience (ratio of recovered strain at 20 minutes to total strain) vs. time of loading.

permanent deformation against stress time. When the "resilient recovery" (*i.e.*, ratio of the amount of recovered deformation at any time during recovery of the total recovered deformation) *e.g.*, at 10 seconds is examined, the relationship with stress time (Figure 4) tends to parallel that of the resilience with the stress time. Similar curves are found for the resilient recovery at other times (*i.e.*, 5, 20, 40, 60 seconds).

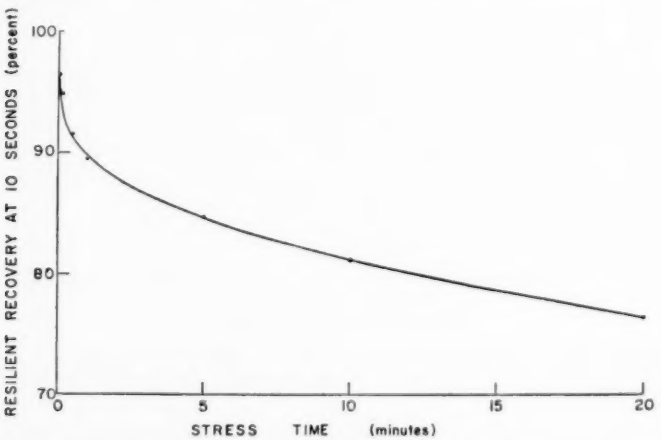

Figure 4. Resilient recovery (ratio of strain recovered after a particular time to recovered strain at 20 minutes) at 10 seconds vs. time of loading.

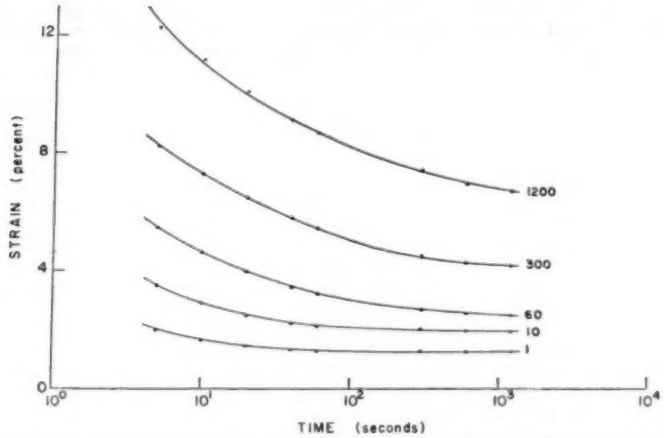

Figure 5. Amount of strain in recovery (for indicated loading times) vs. logarithm of time of recovery.

A comparison of the rates of gel recovery after varying times of stress is given in Figure 5. With shorter periods of stress application, recovery is much more rapid than with longer periods. Additionally, with shorter periods of stress, recovery appears virtually to be completed within 20 minutes, whereas with longer stress periods, further recovery apparently will occur after 20 minutes.

Retardation times may be shown graphically by the following method: on a graph of strain against time of stress are plotted the values of the total strain during recovery for various intervals (*e.g.*, 5, 10, 20, 40, 60, etc. seconds). The time required for the gel to recover to a certain value of strain may then be plotted against the time of application of stress (Figure 6). This figure shows that with increasing time of appli-

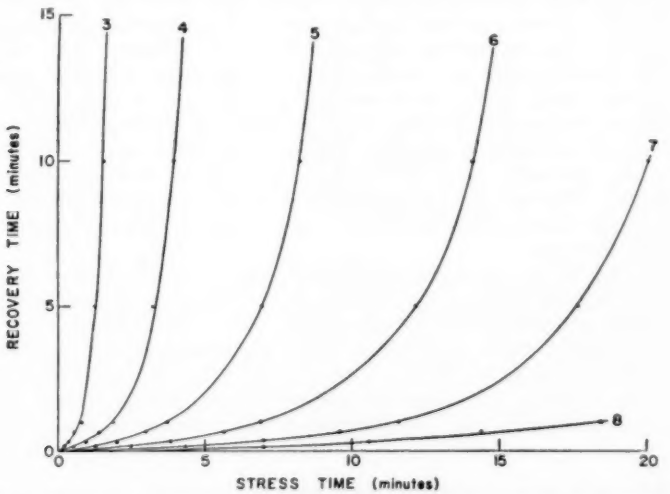

Figure 6. Time to recover to indicated values of total strain vs. time of loading.

cation of stress, the time of retardation of the strain (*i.e.*, time for the gel to recover to a certain value of strain) is greatly increased. Also, for any given stress time, the retardation time increases very markedly in attaining successive increments of recovered strain. This increase is not of the form required by the simple Kelvin (Voigt) body *(1, 9)*, in which $\sigma = \sigma_0 \exp\left(-\frac{t}{T_{ret}}\right)$ (where σ is the strain at a time t, σ_0 is the initial strain, and T_{ret} is the retardation time), since the plot of t against ln σ is not at all rectilinear.

Several points of interest are to be noted in the second series of experiments (Table 2)[b]. One feature is that upon loading the gel a second time, the total strain reached in 1200 seconds is greater than the initial total strain for I_∞ but is *less* for I_{1200_∞}. A second datum of note is the extent of recovery. The permanent strain for the second recovery is 19% greater than that of the first for I_{1200_∞}, but it is almost 200% greater for I_{80_∞}. A similar result is observable in the resilience: the resilience of the second recovery with I_{1200_∞} is 93.4% (compared to 69.9% for the initial recovery) whereas with I_{80_∞} it is 81.3% (compared to 90.6% for the initial recovery). In addition, the amount of deformation produced in the second loading (as based on the first recovery) is less (18.23%) for I_{1200_∞} than that (22.47%) for I_{80_∞}. It should be noted also that in the second loading of I_{1200_∞}, the strain attained in 4 minutes remained at a *constant* value for the remainder of the loading period. This did not occur in the case of the initial loading (where deformation increased throughout) or in either first or second loadings of the I_∞ specimens.

DISCUSSION

The non-linear relationship of permanent deformation with time of application of stress clearly negates the assumption of a viscous element, serially connected, in the model postulated earlier *(11)*. Such an element is deficient in accounting for the strain/time relationship here. It is thus obvious that laminar flow comprises at best a negligible part of the unrecovered strain and that the tentative "viscous" relationship shown previously is merely coincidental. This conclusion then substantiates the suggestion that the permanent deformation is compounded mostly of breakage of junction points, rearrangement of brush-heap molecules, and perhaps establishment of new junctions in the network of polysaccharide molecules.

Similarly, the supposition of a second viscous element, which is connected in parallel with an elastic element, lacks support in the present experiments. Thus, if the gel were analogous to such a model, the time of recovery to a particular value of strain would be unaffected by changes in the time of application of stress; but changing the time of stress application does markedly alter the time of recovery in these starch jellies (Figure 6). Whether it is conceptually heuristic to apply here the idea of a spectrum of retardation times *(1)* (*i.e.*, times of recovery) is not immediately apparent.

The existence of time effects, although not necessarily supporting the concept of ideally viscous flow, does indicate frictional resistance among the movable components of the gel system. Let it be assumed, that with longer periods of stress application (*e.g.*, 20 minutes) there is a certain amount of disruption of the gel network. Then, on the re-application of load after recovery, any temporal retardation in the attainment of the previous amount of strain would suggest the presence of some type of

[b] Although the values in this table differ somewhat from those of Table 1, they are within the range of experimental error.

TABLE 2

Percentage strain and resilience in repeated loading (50 gm.)

Time of initial stress (seconds)	Strain (%)				Resilience (%)			Young's modulus ($\times 10^8$ dynes/cm^2) Stress/recovered strain	
	At initial loading	At 1st 1200 second recovery	At 2nd 1200 second loading	At 2nd 1200 second recovery	At 1st 1200 second recovery	At 2nd 1200 second recovery			
						On basis of original length	On basis of 1st 1200 second recovery	1st loading	2nd loading
60	23.03	2.14	24.60	6.39	90.6	72.3	81.3	1.48	1.67
1200	25.62	6.49	24.76	7.70	74.7	69.9	93.4	1.58	1.79

internal friction (see also *3*). Since there is such a retardation of strain, frictional resistances can be operative, probably involving polysaccharide molecules with their associated shells of sugar and water molecules. (Polysaccharide molecules contributed by the starch alone would be about 22 Å apart if evenly spaced).

One item of importance in interpreting molecular constitution is the correspondence of the three curves: remaining strain, resilience, and resilient recovery in 10 seconds, all considered with respect to the time of loading. Together, these three relationships show that an initially elastic response simply "disappears" in the space of the first few seconds of loading. A slower rate of disappearance is characteristic of longer periods of stress. If the elements of this elastic response were due solely to the energy changes involved in molecular straightening and coiling, this elasticity would not be lost [see the Halton–Scott Blair *(4)* model]. Hence, the molecules constitute part of a network, which latter is disrupted when stress is applied (as suggested above, by breakage of micellar bonds, displacement of brush-heap elements, or crystallization).

The above hypothesis is validated also by the consideration of the relationship between time of retardation and time of application of stress. Increasing retardation time during recovery, following increasing loading time, points to increased internal friction and/or decreased restoring forces. Increased friction could be promoted by changes in position of loose starch and dextrin molecules or loose ends of molecules; decreased restoring forces could result from breakage of original bonds and establishment of new bonds by molecular association. All these effects may conceivably be time-dependent (see further, below).

If the restoring forces (involved in returning the straightened starch molecule to more random and coiled positions) are not as great as in rubber, it is quite possible that long-term loading is not essential for permanent molecular alignment in the starch gel but that even short-term stressing could bring about increased parallelization, *i.e.*, crystallinity. (The electron micrographs of Tachibana, *et al. (12)* show such production of parallel fibrils from a heterogeneous order.) Press *(8)* has suggested the dependence of amount of crystallinity on time of loading in viscose rayon, and the same idea is implicit in Treloar's *(13)* work on rubber.

Repeated application of stress in various materials often results in an increase in elastic modulus *(9)*. This phenomenon has been referred to as "work-hardening" *(10)*. Leaderman *(5)* uses the phrase, "mechanical conditioning," to describe the lack of permanent deformation in loading experiments subsequent to the first loading. In terms of molecular change, Mark *(6)* attributes the change in elastic modulus to crystallization, which he designates as "phase transition."

When the starch gel is loaded repeatedly, the modulus increases from the first to the second loading. Since the strain attained in the second loading of I_{1200} is constant after 4 minutes and does not reach that of the first loading, whereas the strain in the second loading of I_{60} is not constant and is greater than that of its first loading, a definite reinforcement has occurred in I_{1200}. This reinforcement is not interpretable on the basis of the higher percentage of stronger bonds which remain or on the basis of new positions of brush-heap molecules (since these have permitted the

greater initial strain of the first loading). Only the formation of new bonds in the material can be responsible for this phenomenon, which implies increased crystallinity.

However, because the gel is not completely "mechanically conditioned" (*i.e.*, it still shows some permanent deformation) at the second recovery of I_{1200}, some further displacement and crystallization probably occur during the second stressing. Much more of this series of putative changes occurs in the second stressing of I_{60}. Therefore, not only crystallization and the migration of molecules and molecule ends (presumably a viscosity phenomenon) but probably also the gradual rupture of successive micellar junctions are time-dependent phenomena.

A final aspect of the time relationship should be mentioned. It has been shown (Figure 2) that on a logarithmic scale, the permanent deformation increases more rapidly than the total deformation at longer times of loading. The earlier study *(11)* referred the longer-term total deformation in part to crystallization on the basis of its linear form *(7)*. The reconciliation of the two curves can be made either by assigning a significant amount of network disruption to the longer loading time or by assuming that increasing crystallization results in a greatly increased immobilization of the molecules of the deformed gel. The extent to which crystallinity plays a role in the behavior of starch gels under stress should be ascertained by other methods.

SUMMARY

When time of loading of a starch gel is varied, simple mechanical models are inadequate for the interpretation of rheological behavior. Crystallization, or the formation of new micellar bonds, seems to account in part for the departure from idealized models, but displacement of isolated and lightly attached polysaccharide molecules ("brush-heap") and breakage of weaker bonds are probably involved also.

With increased time of stress application, there is an increase in the time of retardation of the strain and in the permanent deformation of the gel. The resilience decreases with increased loading time. When loadings are repeated, the gel shows a higher elastic modulus and decreased permanent deformation and hence demonstrates "work-hardening."

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SOME CHEMICAL AND ORGANOLEPTIC CHANGES IN GAMMA IRRADIATED MEATS[a, b]

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(Manuscript received May 19, 1956)

Preservation of meats by ionizing radiation is technically feasible and potentially a commercial method. It has been shown, however, that undesirable chemical and organoleptic changes occur during irradiation *(6, 8)*. Study of changes in irradiated meats affecting the nutritive value and the consumer acceptance is important for proper selection of meats suitable for radiation and the conditions of radiation.

Peroxide values and carbonyl concentration are good chemical measures of oxidative fat rancidity. The determination of the alkaline pyridine hemochrome concentration is the most convenient means of measuring total iron protoporphyrin. Oxymyoglobin in red meats and carotenoid pigments in some fish and poultry are of importance from a color acceptance standpoint and can be measured accurately from their absorption spectra. The retention of B-vitamins and vitamin E are criteria of nutritive value. The activity of the succinoxidase enzyme system of meats is important because it is a major surviving enzyme consuming oxygen *(9)*. Expressed drip is a well accepted physical method for estimating texture changes. Flavor, which is the major factor affecting acceptance of irradiated meats, must be evaluated by organoleptic techniques.

The formation of peroxides in lipids during radiation has been demonstrated *(10, 16, 18)*. The reduction of preformed lipid peroxides by radiation in an inert atmosphere has been reported *(8)*. Riboflavin in milk was shown to be moderately sensitive to radiation *(3)*. Chemical assays for vitamin E in irradiated butterfat gave high values whereas bioassays showed over 80% destruction of the vitamin *(3)*. Meat pigments were shown to be less susceptible to oxidative changes when meats were radiated in an inert atmosphere. It has been pointed out that during radiation the myoglobin of meat may be converted to brown metmyoglobin, to red oxymyoglobin or to a green pigment *(7)*. However, Huber *et al. (13)* reported that the brown pigment of irradiated meat was different from metmyoglobin and that the red pigment was different from oxymyoglobin.

The enzyme phosphatase was only slightly inactivated by radiation *(3)*. Bacon and corned beef radiated at 1.5 megarep (10^6 rep) were free of off-flavor after cooking *(4)*. Organoleptic studies on chicken meat radiated at 2 megarep gave variable results *(19)*. Hannan *(11)* reported that

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[a] Presented at the Sixteenth Annual Meeting of the Institute of Food Technologists, St. Louis, Missouri, June 13, 1956.

[b] This paper reports research supported by the Quartermaster Food and Container Institute for the Armed Forces and has been approved for publication. The views and conclusions in this paper are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

irradiated beef developed an unpleasant odor and flavor. With radiation doses of 2 megarep no marked change in texture was observed.

It is the purpose of this study to define these major chemical and organoleptic changes discussed here in a wide variety of irradiated meats, including poultry and fish.

EXPERIMENTAL

Meats and irradiation. In Table 1 are listed some of the characteristics of the meats used in this study. The meats were ground fine, thoroughly mixed and packed in suitably enameled No. 2 cans. The filled cans were evacuated and gassed with nitrogen or oxygen through a small hole, and then soldered shut. The canned meat was frozen in a −18° C. room. The cans were shipped in solid carbon dioxide by air

TABLE 1

Characteristics of meats used in irradiation studies

Kind	Atmosphere during irradiation	Description	Water	Fat
			%	%
Beef	O_2	fresh, bottom round	59	21 I.N.[1] 46
Beef	N_2	fresh, round	63	10
Pork	O_2	fresh, from paired uncured hams	48	38 I.N.[1] 63
Pork	N_2	fresh, loin	54	32
Chicken	N_2	fresh, whole	52	14
Salmon, Chinook	N_2	fresh, whole	70	8
Halibut	N_2	fresh, whole	80	....
Tuna, yellow fin	N_2	frozen, whole	75	....
Ham	N_2	quick-cured, 15 lb. ham	52	28

[1] Iodine number.

freight to the Materials Testing Reactor, Arco, Idaho. Samples were thawed, radiated, refrozen and return shipped at solid carbon dioxide temperatures. The samples were held in the frozen state until analysed.

Measures of lipid oxidation. Using a mortar and 100 ml. isooctane, lipids were extracted from 10 g. of ground meat. Peroxide values were determined on a 25 ml. aliquot of isooctane extract by reacting with 50 ml. of acetic acid and 2 ml. of saturated potassium iodide in methanol under nitrogen for 20 min. Released iodine was titrated with 0.01 N sodium thiosulfate. The concentration of carbonyl compounds was determined on a 1 ml. aliquot of the isooctane extract by the method of Henick, Benca, and Mitchell *(12)*.

Measurement of pigments. Hematin pigment concentration was determined on an aqueous extract by the alkaline pyridine hemochrome method of Keilin and Hartree *(15)*. Identity of the hematin pigments was determined by recording the complete spectra from 350 to 650 mμ with a Beckman DR recording spectrophotometer. The ratio of the absorbance at 580 mμ (MbO_2 maximum) to absorbance at 565 mμ (MbO_2 minimum) was used to estimate the amount of oxymyoglobin. The nitrosohemochrome pigment of cured ham was determined by the method of Anderton and Locke *(1)*. Carotenoid pigments of beef and chicken were determined by extraction with acetone, transferring the pigment to petroleum ether, drying over anhydrous sodium sulfate and making absorbance measurements with an Evelyn Colorimeter equipped with a 440 mμ filter. For measurement of the carotenoid pigments of salmon, 50 g. of salmon was homogenized 15 seconds with 100 ml. acetone and quickly filtered. Visible spectra of the acetone extract were recorded using a Beckman DR spectrophotometer.

Assay of vitamins. Tocopherols were determined by a modification of the method by Kaunitz and Beaver *(14)*. Microbiological assays for riboflavin were made using the method given by Snell and Strong *(23)*. Microbiological assays for niacin were

carried out using the method given in *Methods of Vitamin Assay (2)* and assays for thiamine were made using the method of Sarett and Cheldelin *(21)*. Thiamine was determined fluorimetrically by the method given in *Methods of Vitamin Assay (2)*.

Organoleptic evaluation. Organoleptic evaluation of meat samples was conducted using a multiple-comparison method. This method included presenting each judge with a labeled control and 5 coded samples (1 "blind control" and 4 treatment levels). Most of the 12 judges who participated had tasted irradiated meat samples previously and all had extensive experience in evaluating other foods for flavor, color, texture, etc. Judges scored each coded sample for the "difference" between it and the labeled control. Numerical values were assigned to the "difference" descriptive terms; i.e. None—0; Slight—1, 2; Moderate—3, 4, 5; Large—6, 7; Extreme—8, 9. The significance of the effect of treatment as compared with the "blind control" was determined by the "F ratio" calculated by the analysis of variance.

Enzyme activity. Succinoxidase activity measurements were made using a modification of the method by Schneider and Potter *(22)*.

Measurement of drip. Expressed drip was measured by placing a 25 g. sample of meat between two squares of paper toweling, wrapping in a canvas press cloth and placing under 150 p.s.i. for three 5-minute periods using a Carver Laboratory Press.

Water, fat and iodine No. Water content was determined by freeze-drying for approximately 24 hrs. Freeze-dried meats were ether extracted in the Soxhlet apparatus for 19 hrs. to extract the fat. Iodine numbers were determined by bromine absorption *(5)*.

RESULTS

With increased radiation dosage the red color of beef was changed to a dull red and at 30 megarep to a tan color. In Table 2 are shown the chemical changes in ground beef radiated in an atmosphere of oxygen. Peroxide values increased greatly as a function of irradiation dose. Since the peroxide values are above 20 the fat in all samples could be defined as "rancid." The concentration of isooctane soluble carbonyl compounds also increased as a function of radiation dose. These carbonyl compounds were probably formed from the oxidation of unsaturated fatty acids. The porphyrin ring of the hematin compounds was stable to radiation at 0 to 1 megarep but not at 3, 10, and 30 megarep as shown by retention of alkaline pyridine hemochrome. High levels of oxymyoglobin were found in samples radiated to 10 megarep. At 30 megarep there was approximately 100% conversion of oxymyoglobin to metmyoglobin. At radiation doses of 0.3 to 3 megarep—which are of greatest practical concern—riboflavin, niacin, and vitamin E were relatively stable to radiation, whereas thiamine was unstable.

Chemical changes in ground pork radiated in an oxygen atmosphere are shown in Table 3. Peroxide values increased as a function of radiation dose and all irradiated samples had peroxide values above 40 and must be considered "rancid." Compared

TABLE 2

Chemical changes in irradiated beef (O_2)

Irradiation level	Peroxide value	Carbonyl	Hematin compounds		Vitamin retention			
			Alkaline pyridine hemochrome	Oxymyoglobin	B_1[1]	B_2[1]	Niacin[1]	E[2]
r.e.p. $\times 10^{-6}$	Meq./kg. of fat	mM/kg. of fat	%	%	%	%	%	%
0	4	24	100	100	100	100	100	100
0.3	26	21	100	90	....	....	100	....
1	34	39	93	90	76	90	100	....
3	42	53	76	80	16	90	70	63
10	49	50	57	100	6	90	74	....
30	47	55	28	~0	3	63	45	21

[1] Microbiological assay.
[2] Chemical assay.

TABLE 3

Chemical changes in irradiated pork (O_2)

Irradiation level	Peroxide value	Carbonyl	Hematin compounds		Vitamin retention[1]		
			Alkaline pyridine hemochrome	Oxymyoglobin	B_1	B_2	Niacin
r.e.p. $\times 10^{-6}$	Meq./kg. of fat	mM/kg. of fat	%	%	%	%	%
0	3	8	100	80	100	100	100
0.1	41	10	85	60	100	100	100
0.3	50	22	86	70	100	100	93
1	41	20	79	80	84	100	93
3	91	25	72	100	12	65	78
10	95	40	80	60	34	83	78
30	83	47	74	~0	1	61	55

[1] Microbiological assays.

to irradiated beef, irradiated pork showed higher peroxide values undoubtedly because of greater unsaturation in its lipids. The content of carbonyls increased to 5 times the control while that in beef increased to only 2 times that of the unirradiated control. Hematin compounds in irradiated pork showed approximately the same stability as in irradiated beef. The percentage of oxymyoglobin was relatively high in samples radiated up to 10 megarep. Riboflavin and niacin were relatively stable to radiation and again thiamine was labile.

Radiation-induced changes in ground beef radiated in a nitrogen atmosphere are shown in Table 4. Radiation of beef produced insignificant increases in peroxides and carbonyls. This is in contrast to the large increases in beef radiated in an oxygen atmosphere. Destruction of hematin pigments was an approximate function of radiation dose. The red oxymyoglobin pigment was relatively stable to radiation up to 8 megareps. The carotenoid of beef lipids, beta-carotene, was labile to radiation. Vitamin E retention in beef samples radiated in nitrogen was greater than for samples radiated in oxygen. The lability of thiamine in beef radiated in nitrogen was of the same order as beef irradiated in oxygen. In comparison, beef, electron radiated at a level of one megarep, showed a thiamine retention of 38%. Succinoxidase activity was reduced to 16% by a radiation dose of 2 megarep. Texture changes as shown by expressed drip were small.

Chemical changes in pork radiated in a nitrogen atmosphere are shown in Table 5. Just as in the case of beef, pork radiated in a nitrogen atmosphere showed only small increases in peroxides and carbonyls. The increase in red color observed in irradiated

TABLE 4

Chemical and physical changes in irradiated beef (N_2)

Irradiation level	Expressed drip	Hematin compounds		Vitamin retention			Carotenoid retention	Succinoxidase retention
		Alkaline pyridine hemochrome	Oxymyoglobin	B_1 Microbiological	B_1 Fluorimetric	E		
r.e.p. $\times 10^{-6}$	%	%	%	%	%	%	%	%
0	35.2	100	100	100	100	100	100	100
0.7	.....	97	80	....	....	....	....	....
1	.....	87	80	49	80	....	57	25
2	.....	88	80	29	44	100	34	16
4	36.4	61	70	14	10	....	24	12
8	38.4	51	70	....	....	....	....	9
16	39.5	44	50	....	....	....	....	1
34	.....	43	~0	....	....	95	....	....

TABLE 5

Chemical changes in irradiated pork (N_2)

Irradiation level	Oxymyoglobin	Thiamine retention		Succinoxidase retention
		Microbiological	Fluorimetric	
r.e.p. × 10^{-6}	%	%	%	%
0	50	100	100	100
0.7	100	....	....	....
1	100	35	39	98
2	70	25	26	42
4	80	9	11	0
8	80	....	....	0
16	50	....	....	0
34	~0	....	....	....

meat is shown by the increase in percentage of oxymyoglobin at the radiation dose of 1 megarep. Retention of thiamine was about the same level as found in irradiated beef. Both the microbiological assay and fluorimetric determination for thiamine showed good agreement. Succinoxidase activity was decreased to an insignificant rate at 4 megarep, whereas in irradiated beef there was low activity even in the 16 megarep sample.

Radiation of chicken in a nitrogen atmosphere produced a bright oxymyoglobin pink coloration. At high radiation doses a mushy texture and a nauseating odor developed, and gases containing carbon dioxide were produced. In Table 6 are shown the chemical and organoleptic changes in irradiated chicken.

An organoleptic evaluation indicated significant differences between the control and irradiated samples. Peroxides and carbonyls were in low concentration as might be expected for radiation in a nitrogen atmosphere. The carotenoid of chicken fat, mainly lutein dipalmitate, was relatively labile to irradiation. Thiamine retention was of the same order as in irradiated beef and pork.

Salmon radiated at a dose of 2 megarep in a nitrogen atmosphere was bleached and had a metallic and burned odor. This severe bleaching of carotenoids was found also in electron radiated samples at 0.5 and 2 megarep. Irradiation-induced changes in ground salmon which was radiated in a nitrogen atmosphere are shown in Table 7. In organoleptic tests both flavor and color of irradiated samples were shown to be significantly different from control samples. The destruction of carotenoid pigments, mainly astaxanthin, was a function of radiation dose.

Gross observations of irradiated halibut did not show any color, texture or large odor changes. In Table 8 are given the thiamine and organoleptic changes in halibut radiated in a nitrogen atmosphere. In organoleptic tests flavor but not color of irradiated samples was significantly different from the controls.

TABLE 6

Chemical and organoleptic changes in irradiated chicken (N_2)

Irradiation level	Organoleptic	Peroxide value	Carbonyl	Oxymyoglobin	Carotenoid retention	Thiamine retention [2]
r.e.p. × 10^{-6}	Av. score	Meq./kg. of fat	mM/kg. of fat	%	%	%
0	6.2	4.7	0.2	50	100	100
0.7	4.6 [1]	11.9	1.4	80	....	65
1	4.9 [1]	5.2	2.8	70	42	61
2	3.5 [1]	....	....	100	28	42
4	2.4 [1]	0.4	3.5	70	5	17
8	....	0.7	4.3	80	....	....
16	....	....	....	60	....	....
34	....	3.0	....	~0	....	....

[1] Significant at 5% level.
[2] Fluorimetric determination.

TABLE 7

Chemical and organoleptic changes in irradiated salmon (N_2)

Irradiation level	Organoleptic		Thiamine retention[2]	Carotenoid retention
	Color	Flavor		
r.e.p. × 10^{-6}	Av. score difference	Av. score difference	%	%
0	....	....	100	100
0.2	....	....	....	32
0.5	6.6[1]	2.7[1]	....	12
1	7.2[1]	3.8[1]	46	19
2	7.6[1]	4.3[1]	16	2
4	7.6[1]	6.1[1]	3	3

[1] Significant at 1% level.
[2] Fluorimetric determination.

TABLE 8

Thiamine and organoleptic changes in irradiated halibut (N_2)

Irradiation level	Organoleptic		Thiamine retention[2]
	Color	Flavor	
r.e.p. × 10^{-6}	Av. score difference	Av. score difference	%
0	....	....	100
0.5	2.2	1.6[1]	....
1	0.5	2.9[1]	14
2	1.1	3.8[1]	6
4	1.9	5.3[1]	0

[1] Significant at 1% level.
[2] Fluorimetric determination.

TABLE 9

Chemical and organoleptic changes in irradiated tuna (N_2)

Irradiation level	Organoleptic		Oxymyoglobin	Thiamine retention[2]	Succinoxidase retention
	Color	Flavor			
r.e.p. × 10^{-6}	Av. score difference	Av. score difference	%	%	%
0	....	....	~0	100	100
0.2	....	....	30	79	....
0.5	2.2[1]	2.2[1]	30	64	....
1	0.5	3.1[1]	100	35	30
2	2.1[1]	3.9[1]	30	11	30
4	1.7	5.2[1]	30	4	70

[1] 1% level of significance.
[2] Fluorimetric determination.

Tuna irradiated at 2 megarep had a desirable pink color but an undesirable burned odor. Chemical and organoleptic changes in tuna radiated in a nitrogen atmosphere are shown in Table 9. Organoleptic tests indicated that both color and flavor of irradiated samples were significantly different from controls. The high concentration of oxymyoglobin at the dose of one megarep is in agreement with visual observations of the intense pink coloration of irradiated tuna meat. The apparent increase in succinoxidase activity at the dose of 4 megarep may be in error because of the low levels of surviving succinoxidase in the original frozen tuna.

Irradiated cured ham showed a slight decrease in color but not a perceptible odor change at a dose of 2 megarep. Irradiation-induced changes in ham radiated in an atmosphere of nitrogen are shown in Table 10. Flavor and color of irradiated samples

TABLE 10

Chemical and organoleptic changes in irradiated ham (N_2)

Irradiation level	Organoleptic		Nitrosohemochrome retention	Thiamine retention[1]
	Color	Flavor		
r.e.p. $\times 10^{-6}$	Av. score difference	Av. score difference	%	%
0	....	....	100	100
0.2	....	....	76	82
0.5	2.3[3]	1.0[2]	90	71
1	3.4[3]	1.7[3]	90	51
2	4.1[3]	2.0[2]	45	18
4	6.2[3]	4.5[3]	34	6

[1] Fluorimetric determination.
[2] Significant at 5% level.
[3] Significant at 1% level.

were significantly different from controls. Destruction of the nitrosohemochrome pigment was shown to be relatively high at the dose of 2 megarep. Thiamine retention was about the same order of magnitude as the other meats studied.

DISCUSSION

In both beef and pork which were radiated in an oxygen atmosphere large amounts of lipid peroxides and carbonyls were formed. This development of oxidative fat rancidity in meats radiated in oxygen atmospheres is undesirable nutritionally and also undesirable from the standpoint of consumer acceptability. When beef, pork, and chicken were irradiated in nitrogen atmospheres no significant increase in the levels of peroxides were found. Also carbonyls did not increase greatly. This difference between meats radiated in oxygen and nitrogen clearly shows that radiation of meats should be carried out in an inert atmosphere or in vacuum. Under practical conditions it will be easy to obtain canned fresh meat in an oxygen free atmosphere. If fresh meat is vacuum packed prior to irradiation its respiratory enzymes, particularly the succinoxidase system *(9)*, will act as an oxygen scavenger and will remove all oxygen from the container.

All of the irradiated meats evaluated organoleptically in this study were shown to be less acceptable than a non-irradiated control. These organoleptic changes in irradiated meats certainly present the most formidable problem in the acceptability of the radiation of meats.

Since the respiratory enzymes in the canned fresh meat control use up all available oxygen, they cause a deoxygenation of the myoglobin, and at the low oxygen partial pressures which exist some of the myoglobin is oxidized to metmyoglobin. This reaction was most pronounced in the highly oxidizing meat of the tuna, and the control tuna was entirely converted to metmyoglobin. In some of the meats radiated in a nitrogen atmosphere, particularly tuna and pork, there was a conversion of the brown metmyoglobin to the desirable pink color of oxymyoglobin. This formation of oxymyoglobin from metmyoglobin by radiation in nitrogen atmospheres has been definitely confirmed by absortion spectra and other chemical tests. This conversion of metmyoglobin to oxymyoglobin could involve either a reduction of the ferric iron of metmyoglobin to ferrous iron of myoglobin and the subsequent oxygenation of this myoglobin to oxymyoglobin or to the formation of oxymyoglobin by the reaction of hydroxyl radicals with

metmyoglobin. Of these two possibilities the first appears untenable because insofar as is known there is no oxygen for oxygenation of reduced myoglobin to oxymyoglobn. Studies based on the second proposed mechanism are in progress. Thus, aside from loss of nitrosohemochrome in cured meats, radiation-induced changes in hematin pigments do not appear to present critical problems.

The carotenoids of the meats studied are of interest primarily from the view of consumer acceptance. Losses of carotenoid in irradiated chicken and especially in irradiated salmon are undesirable quality changes. The drastic color change in gamma or electron radiated salmon will undoubtedly exclude the fish from radiation processing. Three vitamins for which meats (muscle) are a most important source are thiamine, riboflavin and niacin. Riboflavin and niacin were shown to be relatively stable to irradiation in both beef and pork, whereas thiamine has been shown to be very labile to ionizing radiation in all meats, including chicken and fish, studied. Literature reports indicate that thiamine in meat is also labile to heat processing. For example, ham and pork luncheon meat, 12 oz. cans, processed at 111°C. for 60 min. had a retention of 68%. Pork luncheon meat, 2.5 lb. can, processed at 112° C. for 146 min. had a retention of 52% *(20)*. Chicken, No. 2 can, processed at 121° C. for 70 min. had a retention of 33% *(17)*. The retention of thiamine in ham and pork radiated at sterilization doses is lower than for heat processed ham and pork. However, thiamine losses in chicken radiated at sterilization doses are of the same order of magnitude as in heat processing. These studies also indicate that thiamine is as labile in electron irradiated beef as in the gamma irradiated product.

Loss of thiamine in irradiated meat, particularly in pork products where thiamine is in high concentration, is a very undesirable nutritional change. The destruction of thiamine reported here is the greatest nutrient change recorded to date which is caused by radiation of meats. As stated previously, it has been reported that the chemical test for vitamin E gives high values when applied to irradiated meat whereas bioassay shows losses of vitamin E *(3)*. This might explain our failure to find the radiation sensitivity of vitamin E that other investigators have reported.

The texture changes in irradiated meat were very small as shown by the physical test which was employed to measure drip. Small textural changes resulting from the gamma radiation of meats do not present a problem in the over-all acceptability.

The retention of considerable succinoxidase activity in meat irradiated at sterilizing doses is interesting since the enzyme might act as an oxygen scavenger. In a food container where oxygen is in limited supply chemical reactions involving oxygen which occur before, during or after radiation might be influenced by the oxygen depleting action of this enzyme.

SUMMARY

Some chemical and organoleptic changes in gamma radiated meats were studied. For all meats evaluated organoleptically, the flavor of irradiated meat was significantly different from the un-irradiated. Thiamine in all meats studied was very labile to radiation. Radiation of beef and pork in atmospheres of oxygen and nitrogen show that radiation should be

carried out in an inert atmosphere or vacuum to greatly reduce formation of lipid peroxides and carbonyls. Hematin pigments of irradiated fresh meat were generally bright because of increased oxymyoglobin; however, there was considerable loss of the nitrosohemochrome pigment in cured ham. The carotenoid pigment of salmon was extensively destroyed by irradiation. At the sterilizing dose the succinoxidase system was still active in beef, pork and tuna.

Acknowledgments

The authors wish to thank Marion Simone, Department of Food Technology, for some of the organoleptic evaluations and Barbara Brack, Department of Bacteriology, for the microbiological vitamin assays. James Young of Meridith Fish Company kindly supplied the salmon and halibut for this study and samples of electron irradiated fish.

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EFFECT OF POLYOXYETHYLENE-8-MONOSTEARATE (MYRJ 45) ON LIVER FUNCTION IN PATIENTS CONVALESCING FROM HEPATIC DISORDERS[a]

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(Manuscript received May 26, 1956)

In recent years synthetic emulsifiers of the polyoxyethylene type have found a wide range of uses in the food and pharmaceutical industries *(8, 9)*. Such emulsifiers also have been administered to patients with steatorrheas of various kinds in an attempt to promote intestinal absorption of fat and fat-soluble vitamins *(4)*.

The toxicology of some of these partial ester emulsifying agents, such as polyoxyethylene-20-sorbitan monooleate (TWEEN* 80) and polyoxyethylene-8-monostearate (MYRJ* 45), has been studied extensively in experimental animals and human subjects *(2, 3, 5, 6)*. Kranitz *et al.* have reported that the oral administration of TWEEN 80 in doses of 4.5 to 6.0 g. per day to more than 100 human subjects for periods up to four years was unattended by clinical evidence of damage to liver, kidneys or hematopoietic system *(5)*. Preston and associates have found a similar absence of toxic effects of sorbitan monostearate (SPAN* 60) polyoxyethylene-20-sorbitan monostearate (TWEEN 60) and of MYRJ 45 in relatively short-term studies involving administration of these substances to infants and children *(10)*.

Studies on certain polyoxyethylene stearates, including MYRJ 45, were reviewed in 1953 by the Food Protection Committee of the Food and Nutrition Board of the National Research Council *(11)*. This report concluded from the then available evidence that the safety of MYRJ 45 for use in foods had not been fully established owing principally to the findings of urinary calculi and mild liver changes in rats and hamsters fed high dietary levels for prolonged periods. This report further emphasized that no evidence of gross or microscopic pathology attributable to MYRJ 45 was found in other species. Recent reports of work originally presented in 1952 *(1, 7)* indicates adverse effects of MYRJ 45 in hamsters, particularly at 25% level in synthetic diets, including diarrhea, genitourinary bleeding and increased mortality.

Reports of other studies, however, have described chronic feeding experiments with rats in which bread containing 15% of polyoxyethylene monostearate (MYRJ 45) on a flour basis (10.25 per cent as fed in the diet) *(2, 3)* or purified diets containing up to 20% *(6)* induced no specific pathology. The latter report concluded on the basis of both nutritional and histopathologic evidence "that no reasonable doubt exists concerning

[a] This investigation was supported in part by a research grant from the Atlas Powder Company, Wilmington, Delaware, and by the Clark Fund.

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the safety of the polyoxyethylene stearates even when fed at levels up to 20% of the diet." When they are added to the diet in relatively small (non-nutritive) quantities, these substances are apparently safe.

The present study was undertaken to explore the possibility that such emulsifiers, consumed in small amounts in food for prolonged periods, might have an injurious effect on the livers of patients with pre-existing liver disease *(11)*. In view of the importance of obtaining further information about the effect of polyoxyethylene emulsifiers on liver function, a study was made of the influence of MYRJ 45 on convalescence from hepatic disease.

METHODS

The subjects were patients in the hospital who were convalescing satisfactorily from hepatic disease. Ten patients were studied: Four had Laennec's cirrhosis of the liver, four had infectious hepatitis, one had subacute hepatitis, and one had hepatitis associated with Schistosomiasis. Only patients who were improving by both clinical and laboratory criteria were selected as subjects. Patients who had been seriously ill at any time during their hospital stay were excluded from the study.

The emulsifier administered in every case was polyoxyethylene-8-monostearate (Myrj 45). Initially, the Myrj 45 was given cautiously in small amounts while the clinical course and liver function tests of the patients were carefully followed at weekly intervals. As it became increasingly evident that the preparation was having no adverse influence on the course of convalescence, the amounts administered were increased and the duration of the period of observation extended.

Standard liver function tests were performed at regular intervals on all the patients. These ordinarily included measurement of the serum bilirubin concentration, the cephalin flocculation index and the thymol turbidity test, bromsulfalein retention, serum albumin and globulin concentrations, and the serum alkaline phosphatase level. In some instances total serum cholesterol and esterified cholesterol levels were measured. In four patients, blood hemoglobin concentration and red cell count also were followed.

The Myrj 45 was administered by mouth in 0.5 g. capsules. Three to 6.0 g. per day was administered in divided doses for periods ranging up to 66 days.

RESULTS AND DISCUSSION

Clinical. No ill effects were observed in any of the patients given Myrj 45. The patients tolerated the material well even when it was given at a rate of 6.0 g. per day. From the clinical standpoint, Myrj 45 administration had no effect on the course of the patient's recovery. Convalescence continued normally in every instance.

Laboratory. The results of the liver function tests are summarized in Table 1. These are divided into the determinations done (a) immediately before Myrj 45 administration and (b) at the end of treatment with Myrj 45. There were no changes in liver function that could be attributed to the administration of the Myrj 45 and, indeed, liver function studies showed gradual improvement consistent with the clinical course in almost every patient in the series. There was no evidence of an adverse effect of Myrj 45 feeding on hemoglobin concentration or red cell count in the patients who were observed from this standpoint.

If Myrj 45 were in any sense hepatotoxic, it could be anticipated that such an effect would be magnified in the patient convalescing from hepatitis or Laennec's cirrhosis. The fact that patients convalescing from hepatic disease tolerated appreciable doses of Myrj 45 well, both from the clinical

TABLE 1

Liver function data in patients receiving polyoxyethylene-8-monostearate (Myrj 45)

	Patient	Diagnosis	MYRJ 45/day (g.)	Duration MYRJ 45 treatment (days)	Total MYRJ 45 consumed (g.)	Serum bilirubin (MG./100 Ml.)	Cephalin flocculation	Thymol turbidity (units) turbidity	Serum albumin (GM./100 Ml.)	Serum globulin (GM./100 Ml.)	Alkaline phosphatase (Bodansky units)	Serum total cholesterol (MG./100 Ml.)	Serum esterified cholesterol (MG./100 Ml.)	Bromsulfalein retention 15 min.	Bromsulfalein retention 45 min.	Hemoglobin R.B.C.
1.	A.G.	Hepatitis (Subacute)	6*	30	165	(a) 3.6 (b) 1.2	Neg. Neg.	1.4	4.8 5.1	4.8 3.9	5.2	180	112	93 51	40 21	15.1/5.05 15.6/4.99
2.	H.B.	Laennec's Cirrhosis	6*	5	24	(a) 4.0 (b) 3.7	++++ ++++	13.7	3.8 4.0	4.9 3.9	7.6 6.1					
3.	C.R.	Laennec's Cirrhosis	6*	66	381	(a) 1.1 (b) 1.5	Neg. Neg.	1.3 3.6	4.9 5.8	3.0 3.8	3.9 2.7	143 155	102 115			9.9/3.10 11.4/4.45
4.	N.G.	Infectious Hepatitis	3	6	18	(a)12.5 (b) 8.6	Neg. Neg.	3.3 2.7	5.6 6.2	3.5 3.7	6.4 6.3	182 206	116 145			15.4/5.36 14.3/4.92
5.	R.E.	Laennec's Cirrhosis	6*	45	255	(a) 2.3 (b) 2.2	+++ ++	4.6 5.3	4.8 4.8	3.5 3.8	18.0 12.1	260 236	165 128	47 35	11	10.8/3.21 12.8/4.29
6.	F.B.	Laennec's Cirrhosis	3	60	180	(a) 1.5 (b) 1.4	Neg. Neg.		3.8	3.0	4.5	244	174	25 21	15 15	
7.	G.R.	Schistosomiasis-Hepatitis	3	14	42	(a) 1.0 (b) 1.3	Neg.	3.6 3.3			10.0 10.2					
8.	E.R.	Infectious Hepatitis	4	23	92	(a) 5.7 (b) 2.8	Neg. Neg.	2.3 3.3	4.6 4.9	3.4 3.1	7.3 2.8					
9.	P.L.	Infectious Hepatitis	3	16	48	(a) 3.9 (b) 2.5	Neg. Neg.	11.0 4.6							28 18	
10.	J.C.	Infectious Hepatitis	4	21	84	(a) 3.3 (b) 2.1	++ Neg.		4.8 4.9	4.0 3.4	13.2 7.6				14 9	

* 3 g. per day administered for several days at the outset.
(a) Immediately before MYRJ 45.
(b) At end of administration of MYRJ 45.

and laboratory standpoints, suggests that this material is benign as far as human liver function is concerned.

SUMMARY

Ten patients convalescing satisfactorily from hepatic disease were given 3 to 6 gm. of polyoxyethylene-8-monostearate (Myrj 45) by mouth every day for periods ranging up to 66 days. These subjects were followed carefully from the standpoint of clinical course and liver function tests.

During and after the period of Myrj 45 administration the patients continued to convalesce normally, both clinically and in terms of liver function.

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ESSENTIAL AMINO ACID COMPOSITION OF PULSES AND RICE

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Chattopadhyay and Banerjee *(2)* determined the protein efficiency values of five varieties of pulses (leguminous seeds) by the rat growth method. This value depends primarily on the essential amino acid composition of the protein. The essential amino acids present in seven varieties of pulses and rice were, therefore, determined by the microbiological assay procedure. Protein contents of pulses and rice were also determined.

Estimation of essential amino acids. Different samples of pulses and rice were finely powdered. One g. of the material was taken in a 150 ml. conical flask, mixed with 10 g. of barium hydroxide and 20 ml. of water, autoclaved for 8 hours at 15 lb. pressure, barium precipitated as barium sulphate with 10 N sulfuric acid, volume made up to 100 ml., filtered, pH of an aliquot of the filtrate adjusted to 6.8 and the solution diluted suitably for the estimation of the ten essential amino acids by the microbiological assay procedure described by Barton-Wright *(1)*. Leucine, isoleucine, valine, and tryptophane were estimated with *Lactobacillus arabinosus;* methionine, phenyl alanine, lysine, and histidine with *Leuconostoc mesenterioides* and threonine and arginine with *Streptococcus faecalis.*

Of the basal media of Barton-Wright *(1)* with the exception of the particular amino acid which was to be analysed, 2.5 ml. were taken in each tube. After additions of standards or prepared hydrolysates containing amino acids, at different levels of concentration, the final volume was made up to 5 ml. with water, the tubes plugged, autoclaved at 15 lb. pressure for 15 minutes, inoculated with organisms, incubated for 72 hours at 37° C. and the acid produced titrated with N/10 sodium hydroxide. Standard curves were plotted with acid production as ordinates and the concentration of the respective amino acids as abscissa and amino acid

TABLE 1
Essential amino acid contents of pulses and rice (g. per 100 g. sample)

	Phaseolus radiatus	Lens esculenta	Phaseolus mungo	Cicer arietinum	Pisum sativum	Lathyrus sativum	Cajanus indicus	Parboiled rice
Leucine	2.10	1.96	2.24	1.28	2.00	2.33	1.82	0.41
Iso-leucine	1.67	1.50	1.60	1.07	1.50	2.17	1.38	0.22
Valine	1.45	1.31	1.52	0.85	1.33	1.75	1.19	0.33
Phenyl alanine	0.97	0.86	1.17	0.71	0.96	1.04	1.73	0.21
Methionine	0.22	0.12	0.33	0.18	0.17	0.14	0.19	0.09
Histidine	0.42	0.39	0.44	0.26	0.45	0.52	0.47	0.09
Lysine	1.57	1.70	1.60	1.02	1.86	1.86	1.46	0.18
Arginine	1.74	1.96	1.64	1.45	2.81	3.13	1.60	0.43
Threonine	0.69	0.91	0.74	0.60	0.89	1.18	0.85	0.20
Tryptophane	0.09	0.08	0.09	0.08	0.05	0.07	0.03	0.04
Protein (%)	17	23	22	19	21	25	21	7

TABLE 2
Essential amino acid contents of pulses and rice (g. per 100 g. protein of the sample)

	Phaseolus radiatus	Lens esculenta	Phaseolus mungo	Cicer arietinum	Pisum sativum	Lathyrus sativus	Cajunus indicus	Parboiled rice
Leucine	12.35	8.52	10.18	6.74	9.52	9.32	8.67	5.91
Iso-leucine	9.85	6.52	7.27	5.66	7.14	8.68	6.57	3.08
Valine	8.53	5.70	6.93	4.47	6.53	7.00	5.67	4.71
Phenyl alanine	5.73	3.74	5.31	3.74	4.56	4.16	8.24	3.00
Methionine	1.28	0.53	1.50	0.94	0.80	0.56	0.89	1.32
Histidine	2.47	1.70	2.00	1.38	2.14	2.08	2.24	1.22
Lysine	9.24	7.39	7.27	5.37	8.86	7.44	6.95	2.51
Arginine	10.24	8.52	7.45	7.63	13.38	12.52	7.62	6.14
Threonine	4.06	3.95	3.36	3.16	4.24	4.72	4.05	2.86
Tryptophane	0.54	0.34	0.40	0.45	0.26	0.27	0.17	0.61

values of samples interpolated. Protein contents of samples were determined by the Kjeldahl method. Results are given in Tables 1 and 2.

DISCUSSION

Pulses studied were found to be rich in essential amino acids. These are present in varying quantities in different pulses. Among them, *Lathyrus sativus* was found to contain the largest amount of most of the essential amino acids. If the total amount of the ten amino acids present in the pulses is taken into account, then the pulses can be placed in the following order of superiority with respect to their amino acid content: *Phaseolus mungo, Phaseolus radiatus, Pisum sativum, Cajunus indicus, Lens esculenta* and *Cicer arietinum*. Protein contents of pulses were found to vary with the different pulses. The value was highest in *Lathyrus sativus* and lowest in *Phaseolus radiatus*. If the essential amino acid contents of samples are expressed in relation to their protein content (Table 2) then the pulses with respect to their essential amino acid contents may be arranged in the following order: *Phaseolus mungo, Pisum sativum, Lathyrus sativus, Cajunus indicus, Lens esculenta* and *Cicer arietinum*. The different pulses should be taken in rotation so that all the essential amino acids will be obtained in proper proportion. Rice is very rich in tryptophane, methionine, phenyl alanine and histidine.

SUMMARY

The ten essential amino acids were estimated in seven varieties of pulses and in parboiled rice microbiologically.

Leucine, iso-leucine, valine, histidine, lysine and tryptophane are present in highest concentration in the protein of *Phaseolus radiatus*. Phenyl alanine, methionine, arginine and threonine are present respectively in highest concentrations in *Cajunus indicus, Phaseolus mungo, Pisum sativum* and *Lathyrus sativus*. Rice is rich in tryptophane, methionine, phenyl alanine and histidine.

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EFFECT OF 2,4,5-TRICHLOROPHENOXYACETIC ACID SPRAY ON ORGANIC ACIDS, PECTIN, AND QUALITY OF CANNED APRICOTS[a]

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(Manuscript received June 18, 1956)

Application of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) to apricot trees at the beginning of the pit-hardening period has been shown to increase fruit size, hasten maturity, reduce preharvest fruit drop, and, under certain conditions, hasten red color development *(7, 8, 9)*. For example, Royal apricots sprayed with 2,4,5-T were induced to mature a maximum of 18 days earlier and were a maximum of 35% larger in volume than unsprayed fruit. Increase in fruit size was the result of a stimulation in growth of the flesh, which was as much as 21% thicker than that of unsprayed fruits. Needless to say, the practice of spraying apricot trees with 2,4,5-T is rapidly being adopted by producers for both the fresh market and for canning. Since a larger proportion of the apricots eventually canned by industry will have been sprayed with 2,4,5-T, it seemed advisable to determine whether or not changes in canning quality might be brought about by the use of this material. This study deals with the quality of sprayed and control apricots canned at different stages of maturity. Changes occurring during maturation in organic acids, pectin, and other constituents that are related to flavor, texture, and quality of the canned products were also investigated.

EXPERIMENTAL MATERIALS AND METHODS

Blenheim apricots. Six Blenheim apricot trees in the Winters district were sprayed on April 13, 1955, at the pit-hardening stage each with 6 gallons of an aqueous solution of 2,4,5-T at a concentration of 100 p.p.m. (0.79 ml. of tri-ethyl amine salt of 2,4,5-T per gallon of water). In the Santa Clara district, where the climate is milder and the period from pit-hardening to maturity is relatively long, a concentration of 25 p.p.m. was used. Apricots were harvested at 3-day intervals at Winters from June 20 to July 6, and at Santa Clara from July 22 to August 3. Pressure test and skin color were used as criteria of maturity.

Canning. For each maturity, about 100 lb. of apricots were washed in tap water, sorted for maturity according to skin color, and halved and pitted in a Perrilli apricot pitter. No. 2½ cans were filled with 19.5 ounces of fruit and 10.5 ounces of 40° Brix sucrose syrup. Cans were sealed in a vacuum closing machine under 15 inches of vacuum and heat processed at 212° F. for 22 minutes in the Anderson-Bangrover rotary cooker. Processed cans were cooled rapidly in water for 7 minutes in a rotary cooler and stored at 68° F. for 3 months.

Pressure test. Firmness of the fresh apricots was measured by a Magness-Taylor pressure tester (16) with a plunger 5/16 inch in diameter, and a pressure range of 0-10 lb. The tester was applied to the peeled area of the cheek side of the fruit, and

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[a] Presented at the Sixteenth Annual Meeting of the Institute of Food Technologists, June 13, 1956, St. Louis, Missouri.

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pressure was applied until the plunger had penetrated 5/16 of an inch. The average reading for 24 apricots from each sample was reported.

Total titratable acidity. Duplicate 10-g. samples of pulped apricots were weighed into 250 ml. beakers, and 200 ml. of distilled water was added. The resulting mixture was tritrated with 0.1 N. sodium hydroxide until the pH value reached 8.0 in the Beckman pH meter. Results were expressed as percentage of malic acid.

Soluble solids. The soluble-solids content of the pulped sample was determined by using a Zeiss Opton refractometer.

Lyophilization of the sample. Contents of 5 cans of apricots were macerated in a Waring blender. Three-hundred g. of slurry was weighed into an enamel pan and lyophilized for 48 hours in a Stokes Freeze Drier under a pressure ranging from 50 to 150 μ of Hg. Weight of the slurry represented by one g. of the lyophilized product was determined. The dried product was stored at 10° F. (−12° C.) in tightly sealed glass bottles.

Malic and citric acid. A 0.3000-0.5000 g. sample of the lyophilized powder was accurately weighed and mixed with 1 g. of silicic acid powder. The mixture was thoroughly mixed with 1.0 ml. of 0.5 N H_2SO_4 and transferred quantitatively to a prepared silicic acid column, 15 mm. i. d. and 25 cm. long. Generally, the column, solvents, and indicator were prepared in the manner described by Bulen *et al. (6)* and Dame *et al. (10)*. The column was packed with a mixture made from 16 g. of dried silicic acid powder and 10 ml. of 0.5 N H_2SO_4. A slight pressure was maintained with compressed air that was bubbled through 6 N NaOH to remove carbon dioxide. Air pressure was adjusted to collect about 60 ml. of effluent per hour. Solvents used in developing the column were 100 ml. each in succession of 10%, 20%, and 30% n-butanol in chloroform, followed by 400 ml. of 35% n-butanol in chloroform (v/v). The first 300 ml. of effluent was discarded. Five-ml. fractions of effluent were collected in 10-ml. test tubes by an automatic constant-volume fraction collector. Each fraction was washed with two 7-ml. portions of distilled water in a 125-ml. Erlenmeyer flask and titrated with 0.02 N NaOH, using two drops of 0.1% phenol red as indicator. Results were reported as percentage of malic or citric acid in the fruit on a dry basis.

Pectin. The method used to determine total pectin, protopectin, and water-soluble pectin was essentially the carbazole method described by McCready and McComb *(17)*. Methods for fractionation of the pectin into protopectin and water-soluble pectin were the same as those described by Postlmayr *et al. (20)*, except that the versene-soluble fraction was not determined but included in the protopectin fraction.

Organoleptic evaluation. A panel of 8 experienced tasters appraised the samples for color, general appearance, texture, flavor, and clearness of syrup. A randomized complete block design was employed. Six samples of control and 2,4,5-T sprayed apricots, canned at different pressure tests, were evaluated once a day for 5 days. The judges were requested to score the samples on a 10-point scale ranging as follows: *excellent*, 9-10; *good*, 7-8; *fair*, 5-6; *poor*, 3-4; and *very poor*, 1-2.

Analyses of variance. Results of organoleptic evaluation were analyzed for variance by the method described by Snedecor *(22)*. Least significant difference (LSD) was calculated by the method described by Sheffé *(21)*.

Trichromatic coefficients. The reflectance readings of the canned apricot halves measured in a three-filter (red, green, and blue) Colormaster differential colorimeter (3) were used to calculate the trichromatic coefficients (chromaticity co-ordinates). The representative apricot halves from five No. 2½ cans for each sample were filled into the cell (7 x 7 x 2.5 cm) with either the pit side or skin side facing the light source. Thirty readings were made on each sample, and the average value was used to determine the color co-ordinates according to the C.I.E. (International Commission on Illumination) system. The following equations were used to calculate C.I.E. tristimulus values from the reflectance readings:

$$X = 0.784\ R + 0.196\ B$$
$$Y = G$$
$$Z = 1.18\ B$$

where R, B, and G, are per cent of reflectance of light when a red, blue or green filter was used. A standard white glass plate was used as a reference.

Trichromatic coefficients in the C.I.E. system were then calculated from the tristimulus values according to the following equations:

$$x = \frac{X}{X+Y+Z}$$

$$y = \frac{Y}{X+Y+Z}$$

Purity, dominant wave length, and brightness of the samples were determined from the trichromatic coefficients according to the Handbook of Colorimetry by Hardy *(13)*.

Syrup viscosity. The syrup was separated from the apricots and centrifuged for 15 minutes at 1750 r.p.m. Time required for 10 ml. of the syrup to flow at 30°C. through the capillary of the Ostwald viscosimeter was recorded with an accuracy to one-tenth of a second. Results were reported as relative viscosity, using distilled water as a reference liquid.

RESULTS

Decrease in total acidity with maturation. Changes in acid content of Blenheim apricots with lowering of pressure tests are shown in Figure 1. Acidity in apricots

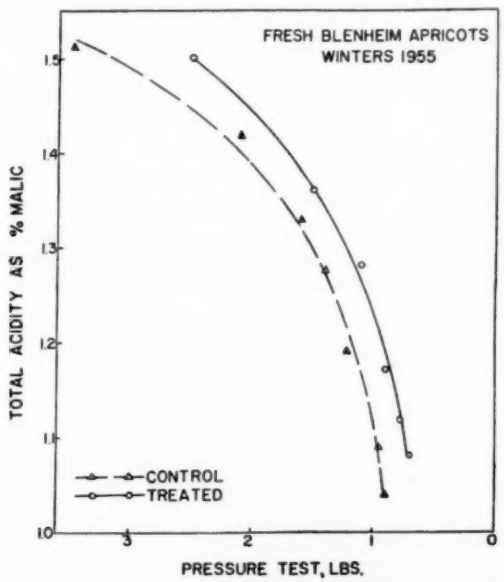

Figure 1. Changes in total titratable acidity during maturation of Blenheim apricots. The treated samples were harvested from trees sprayed with 6 gallons of 2,4,5-T solution (100 p.p.m.) for each tree at the pit-hardening stage.

decreases as they ripen on the tree. This was true for both the control and the sprayed samples. At the same pressure test, the control was slightly lower in acidity than the sprayed sample. Acidity changes were correlated here with pressure test instead of skin color, because 2,4,5-T spray tends to enhance red color development, causing difficulties in differentiating maturity of the control and the sprayed samples by color alone. The use of pressure test to determine maturity is simple and objective. The control sample at average pressure test of 3.5 lb. has a total acid content of 1.52% as malic

acid on the fresh-fruit basis. The sample at 0.84 lb. pressure test has a value of only 1.03%. Within the narrow pressure range of 3.5 and 0.84 lb., a decrease of almost 35% of the total acid has occurred. As will be shown later, acidity is one of the important factors contributing to flavor of canned apricots.

Ratio of soluble solids to total acidity. The soluble-solids content of apricots increases and the total acidity decreases when they mature on the tree. In green fruit the soluble-acids content is lower, but acid content is higher than in matured fruit. By taking the ratio of soluble solids to total acid (S.S./A.), a number is obtained which is even more indicative of the maturity changes. Figure 2 indicates that the

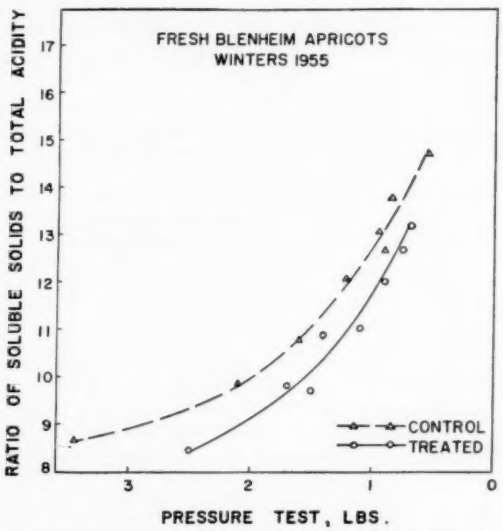

Figure 2. Ratio of soluble solids to total acid of Blenheim apricots at different pressure tests. The treated samples were harvested from trees sprayed with 6 gallons of 2,4,5-T solution (100 p.p.m.) at the pit-hardening stage.

S.S./A. ratio increases as the pressure test of apricots decreases. The trend was the same for the control and the sprayed samples. At the same S.S./A. ratio, the sprayed sample has a lower pressure test than the control sample. When the pressure test decreased to 1 lb. or lower, the values of S.S./A. for the sprayed and untreated control samples tend to approach equality. At this stage, the fruits were overmature.

Malic and citric acids. Preliminary investigation by paper chromatography methods revealed that malic and citric acids were the two organic acids present in significant quantity in canned Blenheim apricots. The amount of the two acids present in apricot samples canned at different pressure tests was determined by silicic-acid gel column chromatography. Results are shown in Figure 3. Malic acid is the dominant organic acid in apricots, although a smaller amount of citric acid is also present. Both malic and citric acid decreased as the presure test of the fresh fruit decreased. The rate of decrease of both acids was faster in the sprayed samples than in the control. Thus, rapid rate of organic acid metabolism might be associated with rapid growth rate of the apricots sprayed with 2,4,5-T. At the same pressure test, the malic acid content of the sprayed sample was lower and citric acid content higher than the control sample. When the pressure test decreased to around 1.1 lb., malic acid in the sprayed samples went up again. When the pressure test decreased further the malic acid content of the

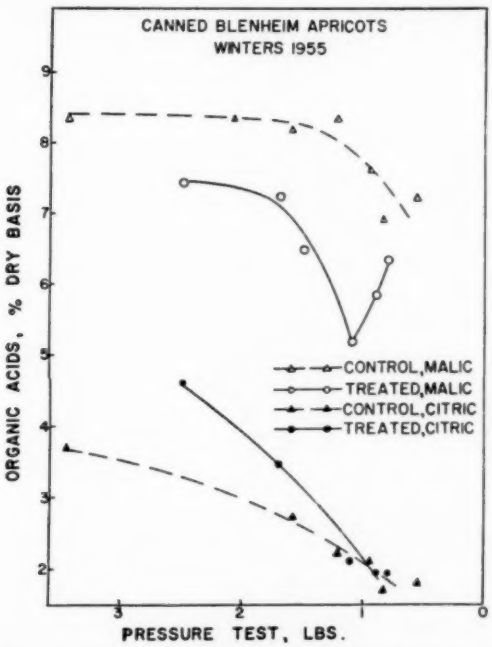

Figure 3. Change in acid contents of Blenheim apricots canned at different pressure tests. The treated samples were harvested from trees which were sprayed with 6 gallons of 2,4,5-T (100 p.p.m.) for each tree at the pit-hardening stage.

sprayed and the control samples approached equality. The same was true for citric acid. At this stage, the fruits were overmature.

Pectic changes accompanying maturation. The pectin contents of Blenheim apricots canned at different pressure tests are shown in Table 1. In both control and sprayed series protopectin decreases and water-soluble pectin increases with decrease in pressure test of the fresh fruit. Total pectin also decreased with decrease in pressure test for the fresh fruit. This might have been caused by hydrolysis of pectic materials by the pectic enzyme polygalacturonase to oligogalacturonides, or pectin of low molecular weights, which were no longer precipitable by the alcohol solution used in this investigation.

It is known that protopectin in plant tissue acts as a binding material between cell walls and is responsible for firm texture of fruit tissues. The softening in texture of apricots during maturation might be explained by the gradual conversion of protopectin to water-soluble pectin. This action loosens the adhesive force between cells, and causes softening of the texture.

Chemical analyses and flavor evaluation. After storage for 3 months at 68° F., both control and sprayed apricots canned at comparable pressure tests were evaluated for flavor by a randonized complete block design method. Chemical analyses were also made after each quality evaluation. Results are shown in Table 2. The panel preferred the flavor of the samples canned at lower pressure tests. The rato of soluble solids to total acid correlated well with the flavor acceptance of the canned product. Samples with higher S.S./A. ratios were preferred over those with lower values. Information gathered in our acceptance laboratory has shown that the optimum S.S./A. ratio for flavor acceptance of canned apricots lies between 35 and 40. Better flavor acceptance

TABLE 1

Pectin content of Blenheim apricots canned at different pressure tests

Pressure test of apricots prior to canning pounds	Total pectic substances		Protopectin			Water soluble pectin		
	Fresh fruit basis %	Dry basis %	Fresh fruit basis %	Dry basis %	% of total pectic substances	Fresh fruit basis %	Dry basis %	% of total pectic substances
(1) Control								
9.8	0.65	6.24	0.50	4.80	76.92	0.15	1.44	23.08
2.6	0.62	3.79	0.28	1.70	44.85	0.34	2.09	55.15
1.4	0.57	3.25	0.21	1.19	36.62	0.36	2.06	63.38
0.51	0.58	3.02	0.15	0.78	25.83	0.43	2.24	74.17
(2) 2,4,5-Trichlorophenoxyacetic Acid (25 ppm)								
7.6	0.62	4.88	0.34	2.68	54.92	0.28	2.20	45.08
2.9	0.62	4.41	0.29	2.10	47.62	0.32	2.31	52.38
1.4	0.71	4.20	0.22	1.28	30.48	0.49	2.92	69.52
0.80	0.66	3.94	0.17	1.01	25.63	0.49	2.93	74.37

of apricots canned at lower pressure test might be explained by the decrease in total acidity and increase in soluble-solids content, thus increasing the S.S./A. ratio to a value near the optimum range. It is important to point out that improvement in flavor was not solely related to the decrease in acidity and increase in soluble solids. Formation of aromatic compounds, softening of texture, decrease of astringent materials, and other biological changes during ripening also contribute to better flavor. At comparable pressure tests, there was a slight preference for the control over the sprayed sample, but the difference was statistically insignificant at the 5% level. The maturity of fresh fruit for canning apparently is an important factor influencing flavor of the canned product. Spraying with 2,4,5-T did not cause any off-flavor provided the concentration and time of spraying and harvest were properly controlled.

Quality evaluation. In addition to flavor, the quality of canned apricots can be evaluated according to color, general appearance, texture, and clearness of syrup. Results shown in Table 3 indicate that color scores went higher as the pressure test of the fresh fruit decreased. This was probably caused by decrease in chlorophyll *(13)* and increase in carotenoids during maturation. General appearance, texture, and clearness of syrup were rated better in samples canned at 2 to 3 lb. pressure test than those canned at 1 lb. Thus, a compromise between flavor and the other factors is necessary for the most desirable product. Results obtained here seemed to indicate that apricots from Winters at 1.4 lb. average pressure test were at optimum maturity for canning. The fruits should be harvested at an average pressure test slightly above this to allow for the ripening while the fruit is transported from orchard to cannery. Spraying with 2,4,5-T seems to hasten color development, but did not cause important differences in general appearance, texture, and clearness of syrup, provided the maturity of the fresh fruit at the time of canning was properly controlled. The syrup viscosity of apricots canned at a firmer stage was higher than those canned at a softer stage, This might be caused by the decrease in length of the polygalacturonide chain of the water-soluble pectin or other unknown factors.

Color coordinates vs. visual color. Color of the canned apricot samples was evaluated with a 3-filter reflectance colorimeter. The trichromatic coefficients and color characteristics of sprayed and control samples are compared with the visual color grades in Table 4. Purity and dominant wave length of the canned product increases and brightness decreases as the pressure test of the fresh fruit decreases. Visual color grade of the canned product also increases with decrease in pressure test of the fresh fruit. Decrease in pressure test of the fresh fruit was accompanied by a decrease in green

TABLE 2

Chemical analysis and flavor evaluation of Blenheim apricots canned at different pressure tests, Winters, 1955

Control					2,4,5-T 100 p.p.m.				
Pressure test of fresh apricots prior to canning, pounds	Brix at 20° C.	Total acid as % malic	Brix to acid ratio	Average flavor score[1] (LSD = 0.51 at 1%, 0.43 at 5% levels)	Pressure test of fresh apricots prior to canning, pounds	Brix at 20° C.	Total acid as % malic	Brix to acid ratio	Average flavor score[1] (LSD = 0.51 at 1%, 0.43 at 5% levels)
3.5	23.29	0.932	24.99	5.32	2.5	22.80	0.866	26.33	**5.44**
1.2	23.32	0.775	30.09	6.68	1.4	23.07	0.809	28.52	6.54
0.84	23.20	0.667	34.78	7.17	0.78	23.10	0.738	31.30	6.68

[1] Average flavor score graded five times by eight judges on a ten point scale, an excellent sample scoring 9-10; good, 7-8; fair, 5-6; poor, 3-4, and very poor, 1-2. LSD is the least difference between samples in order to be significant at the probability level indicated.

TABLE 3

Quality evaluation[1] of Blenheim apricots canned at different pressure tests, Winters, 1955

Pressure test of fresh apricots prior to canning, pounds	Visual color (LSD = 0.73 at 1% level)	General appearance (LSD = 0.80 at 5% level)	Texture (LSD = 0.84 at 5% level)	Clearness of syrup (LSD = 0.73 at 1% level)	Relative viscosity of syrup
(1) Control					
3.5	4.36	6.14	7.00	8.93	66.13
1.2	7.07	7.36	4.29	6.50	33.30
0.84	8.23	3.43	3.29	3.43	15.13
(2) 2,4,5-T 100ppm					
2.5	6.00	6.86	7.00	8.50	40.31
1.4	6.71	5.64	4.13	5.07	21.35
0.78	7.50	5.00	3.25	3.36	17.92

[1] The scores are the average of 40 judgments. With the exception of relative viscosity, each factor was graded on a ten-point scale, an excellent sample scoring 9-10; good, 7-8; fair, 5-6; poor, 3-4, and very poor, 1-2.

and an increase in orange-yellow color. The color of the pit side of the apricot halves was inferior to that of the skin side because purity of the pit side is lower and its brightness is higher.

Comparison of the visual score of samples canned at different pressure tests indicates a definite improvement in color accompanying maturation. The sample canned at 3.5 lb. pressure test still has a little green tint at the shoulder side of the apricot halves, and was graded an average color score of 4.36 points on the 10-point scale. Samples canned at 1.2 and 0.84 lb. were scored 7.07 and 8.23 points respectively. Results are significant at the 1% level. Spraying with 2,4,5-T improves the color of apricots canned at higher pressure tests. In the low pressure range, the sprayed samples were scored lower in color grade than the respective control samples. Probably this was caused by the development of an undesirable deep-brown color in the sprayed sample when the fruit becomes overmature.

DISCUSSION

Acidity in fruit usually decreases with maturation, as indicated by the work of David *et al.* on peaches *(11)*, Dame *et al.* on pears *(10)*, and Allen *(1)*, Atkinson and Strachan *(5)*, and Strachan *et al (23)* on apricots. Results obtained in this investigation demonstrated the rapid decrease in acidity in apricots with maturation in the narrow range of pressure test suitable for canning. Thus, proper control of harvest maturity and post-harvest handling before canning are important factors influencing quality of the canned product. Apricots from different districts may differ in acidity, even at the same pressure test. For example, a sample of Blenheim apricots from Winters has a total acidity of 1.32% as malic acid at a pressure test of 1.5 lb. Another sample of Blenheim apricots from Santa Clara has an acidity of only 0.94% at the same pressure test. This was probably caused by differences in climate, soil properties, and horticultural practices. For quality control purposes, it is important to ascertain the optimum S.S./A. range and the pressure test range, which may vary slightly from district to district and from season to season. Control of the S.S./A. ratio in the canned product might be achieved by (a) controlling the maturity of the fresh fruit, (b) adjusting the concentration of sucrose syrup used for canning, and (c) controlling horticultural practices in the field. For ascertaining harvest maturity, a combination of pressure test,

TABLE 4

Color of canned Blenheim apricots canned at different pressure tests, Winters, 1955

Pressure tests of fresh apricots prior to canning, pounds	Purity %		Dominant wavelength mμ		Brightness %		Trichromatic coefficients				Average visual score[1] LSD[2] = 0.73 at 1% level
							x		y		
	Skin side	Pit side	Skin side	Pit side	Skin side	Pit side	Skin side	Pit side	Skin side	Pit side	Skin side
(1) Control											
3.5	80.4	77.2	580.8	580.0	15.89	17.58	0.4771	0.4670	0.4494	0.4484	4.36
1.2	81.5	80.9	581.6	582.2	16.00	17.27	0.4835	0.4847	0.4474	0.4427	7.07
0.84	83.5	82.4	582.9	583.0	15.44	16.70	0.4948	0.4934	0.4429	0.4409	8.23
(2) 2,4,5-T 100 ppm											
2.5	79.0	76.0	580.1	580.3	16.85	18.82	0.4713	0.4665	0.4504	0.4443	6.00
1.4	80.2	79.0	582.2	582.0	16.89	18.06	0.4843	0.4800	0.4421	0.4403	6.71
0.78	82.0	80.5	582.2	582.3	16.39	17.86	0.4885	0.4848	0.4453	0.4418	7.50

[1] See footnote 1, Table 3.
[2] LSD is the least difference between samples in order to be significant at 1% level.

S.S./A. ratio, and flesh and skin color will give a better understanding of the problem.

Organic acids in apricots have been reported by Nelson *(18)* and Anet and Reynolds *(2)* who found malic acid to be 76% and citric acid 16% of the organic acids in apricots. Small amounts of L-quinic acid and succinic acids were also found in apricots by Anet and Reynolds *(2)*. Since malic and citric acid constitute over 90% of the organic acids in apricots, this investigation of variations in organic acid during maturation deals with the two acids only. The sum of malic and citric acid was found to be higher than the total acidity. This might be explained by the presence of citrate and malate ions in the fruit, which were converted into free acid form by sulfuric acid on the silicic acid column.

Nuccorini and Ducci *(19)* reported that length of ripening period is related to total acid content of the fruit; ripening is more rapid when malic acid is prevalent, less rapid when citric and tartaric acids are prevalent. Thus, both malic and citric acid are involved in the ripening process. Since the rate of metabolism for both malic and citric acid during maturation was more rapid in sprayed than in control samples, it is highly probable that organic acid metabolism is associated with the rapid rate of growth caused by the 2,4,5-T spray.

Recent work on the mechanism of growth in auxin-sprayed fruits *(15)* has indicated that an auxin may form high energy ester bonds with coenzyme A, which suggests an energy-controlling action by the growth hormone. Leopold *(15)* studied the reaction of some organic acids with coenzyme A in the presence of tomato mitochondria, and found that the presence of several auxin componnds could bring about the enzymatic disappearance of the free sulfhydryl groups of coenzyme A. The most active auxins were the most effective (e.g., 2,4-D, indoleacetic acid), weak auxins were less so (e.g., p-chlorophenoxy acetic acid, indolebutyric acid), and compounds closely allied to auxin but lacking auxin activity were ineffective (e.g., cyclohexane acetic acid, 2,4-dichlorbenzoic acid). Adenosine triphosphate and coenzyme A can increase auxin-induced growth in the pea straight-growth test. All these findings point to the theory that auxin may control a high energy bond system in plants through the formation of a high energy phosphate bond on the auxin itself. The result found in this work seems to indicate that the speeded-up organic acid metabolism induced by the 2,4,5-T spray might be related to the stimulation of growth rate.

Growth rate may be influenced by many other factors, such as temperature, amount of light received by the plant, leaf surface available, and availability of water and nutrients. Each factor might be important in determining metabolic rates and thus in controlling growth rate as well. In the Sacramento Valley, as at Winters, where the earliest maturing apricots are produced because of a warmer climate, a higher concentration of 2,4,5-T (100 p.p.m.) is needed to stimulate growth. In the coastal region like Santa Clara, where the temperature is milder and the period between pit hardening and maturation is longer, 2,4,5-T at a concentration of 25 p.p.m. is enough to promote growth.

Whether 2,4,5-T leaves any spray residue or toxic effect in the canned product remains to be investigated. Chemical analysis of residual 2,4,5-T

and feeding experiments are needed to answer this question. Since 2,4,5-T was applied to the tree 2 to 3 months before harvest and the concentration of the spray was very dilute, this growth regulator is not likely to create a serious spray residue problem. Proper control of concentration, time of application and harvest would result in a canned product with quality near or equivalent to the unsprayed sample.

SUMMARY

Blenheim apricot trees at Winters were sprayed with 2,4,5-trichlorophenoxyacetic acid solution (100 p.p.m., 6 gal. per tree) at the beginning of the pit-hardening period to stimulate growth. The apricots were canned at different pressure tests in 40° Brix sucrose syrup according to commercial procedure. The canned products were analyzed for malic and citric acid contents by silica-gel column chromatography and for pectic materials by the carbazole method.

Results indicate that malic acid is the dominant organic acid in apricots, although a smaller amount of citric acid is also present. Malic and citric acid contents decrease as the fruits mature on the tree. Both malic and citric acid decrease more rapidly in the 2,4,5-T sprayed samples than in the controls. Stimulation of apricot growth by 2,4,5-T was thought to be related to the faster rate of metabolism of malic and citric acid in the fruit.

Conversion of protopectin to water-soluble pectin is responsible for softening of the apricot during maturation. The maturity of the fresh fruit at the time of canning has an important influence on the quality of the canned product. Use of the ratio of soluble solids to total acid and the pressure test as objective criteria for maturity is discussed. Among the samples investigated, a pressure test of 1.4 lb. immediately before canning was considered as optimum maturity. With proper control of concentration and time of application as well as time of harvest, spraying with 2,4,5-T could yield a canned product near or equivalent to the quality of an unsprayed sample. Color coordinates of canned apricot samples were determined with a three-filter reflectance colorimeter, and the results correlated with visual color grading. The influence of 2,4,5-T spray and maturity of the fresh fruit on flavor, color, texture, and syrup viscosity of the canned product is presented.

Acknowledgment

The authors wish to express their appreciation to Professor J. C. Crane of the Pomology Department and Mr. Edward Bowles, Farm Advisor at Santa Clara County, for their interest and help in securing apricots for this study.

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BACTERIOLOGICAL FLORA IN FROZEN AND CHILLED ORANGE JUICE[a]

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(Manuscript received June 13, 1956)

Since the introduction of frozen orange concentrate approximately ten years ago, a number of papers have appeared in the literature pertaining to coliforms[b] in this product. Some of the investigators who have contributed knowledge in this field are Beisel *(2)*, Beisel and Troy *(3)*, Brokaw *(5)*, Ferraro and Appleman *(7)*, Hahn and Appleman *(8)*, Martinez and Appleman *(10)*, Patrick *(15, 16)*, Teunisson and Hall *(19)*, Wolford *(23, 24, 25)* and Wolford and Berry *(26, 27)*. The results of some of these investigators have varied considerably. For instance, Martinez and Appleman *(10)* and Ferraro and Appleman *(7)* were unable to isolate coliforms from any of the samples of orange juice examined, whereas Wolford *(25)* isolated 236 coliforms from 79 samples of juice and found 72% of the isolates were *Aerobacter aerogenes*, 19.5% *Escherichia coli* and 8.5% intermediates. Patrick *(16)*, however, selected certain isolates for study and presented evidence to show that of 217 coliforms isolated from orange concentrate and damaged oranges, 64% were *Escherichia coli* types, 19% were *Aerobacter aerogenes* types and 17% were intermediate types.

In order to learn more about the prevalance of certain types of coliforms and related organisms in orange juice, it was decided to determine the identity of some of the Gram negative rods isolated from samples of orange juice by use of Violet Red Bile agar, a selective medium for coliforms.

Data show that of the cultures isolated in this investigation, 65% were *Aerobacter aerogenes* types and 35% intermediate types. No *Escherichia coli* types were isolated. *Aerobacter aerogenes*, *Escherichia freundii*, *Escherichia intermedium*, *Serratia marcescens* and members of the genus Erwinia were isolated from one sample of orange juice.

EXPERIMENTAL PROCEDURE

Isolation of Gram negative rods from orange juice was accomplished by a direct plating method using Violet Red Bile agar, a selective medium for coliforms. All samples were adjusted to pH 6.5 with sterile sodium hydroxide prior to plating. Incubation was at 37° C. for 18 hours.

Of the 150 Gram negative rods involved in this investigation, 82 were isolated from 42° Brix orange concentrate reconstituted to single strength by production methods while the remaining 68 cultures were isolated from experimental samples of orange juice concentrate reconstituted to single strength aseptically in the laboratory.

After the isolates were purified on Levine's Eosin Methylene Blue agar and, with the exception of one culture, found to ferment lactose, Indol production (I), methyl

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[a] Presented before the Sixteenth Annual Meeting of the Institute of Food Technologists, St. Louis, Missouri, June 13, 1956.

[b] The term coliform is used here to designate Gram negative, lactose-fermenting, non-sporeforming rods.

red reaction (M), Voges-Proskauer (V), and utilization of citrate as sole source of carbon (IC), IMVIC characteristics, were determined by tests described by the American Public Health Association *(1)*. The Voges-Proskauer test was also checked by the method of Vaughn and coworkers *(22)*. Other biochemical tests to differentiate coliforms were also carried out according to prescribed procedures. These tests included Eijkman's test at 46°C. (Perry and Hajna Modification) *(17)*, nitrate reduction, lactose fermentation and gelatin liquefaction *(1)*; Vaughn, Levine and Smith buffered boric acid lactose test at 43°C *(21)*; cellobiose, salicin, sucrose, glycerin, starch, and inositol fermentation, hydrogen sulfide production (Peptone iron agar) *(20)*, and utilization of uric acid and uracil as sole sources of nitrogen *(11)*. The hanging drop method was used to determine motility *(12)*.

To differentiate between coliforms and Erwinia, Jones Method as described by Elrod *(6)* was used to test the pathogenicity of the isolates against carrots.

Other tests to determine the identity of 17 cultures isolated from one sample of orange juice were carried out by procedures recommended in the *Manual for Pure Culture Study (12)*. *Bergey's Manual (4)* (Breed, Murray and Hitchens) served as a guide for interpretation of data. *Escherichia coli* ATCC 4157 obtained from American type culture collection, Georgetown University, Washington, D. C.; *Escherichia freundii* and *Escherichia intermedium* obtained from Dr. Philip R. Edwards, Department of Health; Education and Welfare Service, Communicable Disease Center, Chamblee, Georgia; *Aerobacter aerogenes* and *Serratia marcescens* obtained from the Kraft Foods Company, Research Laboratories Stock Culture Collection, were used as controls throughout this investigation.

RESULTS

Some of the culture characteristics of organisms isolated from production and experimental samples of chilled and frozen orange juice by a direct plating method are presented in Tables 1 and 2.

It can be seen that by using Parr's scheme, *(13, 14)* and grouping the 150 isolates according to their IMVIC pattern, they fall into two major groups as follows:

Types	Isolates from production samples		Isolates from experimental samples		Total	
Aerobacter aerogenes	64	78%	33	48.5%	97	65%
Intermediate	18	22%	35	51.5%	53	35%
Total	82		68		150	

Of the 17 Gram negative rods isolated from one sample of orange juice, 5 cultures were identified as *Aerobacter aerogenes*, 4 as *Escherichia freundii*, 2 as *Escherichia intermedium* and one as *Serratia marcescens*. The ability of the remaining 5 cultures to produce soft rot in carrot tissue indicated that these cultures closely resembled the genus Erwinia.

DISCUSSION

Although the data indicate that various types of coliforms and related organisms, i.e., Erwinia, were isolated from chilled and frozen orange juice by use of Violet Red Bile agar, it should be stressed that not all coliforms are considered of fecal origin and significant from a public health viewpoint. The 10th edition of *Standard Methods for the Examination of Water and Sewage* lists only those organisms having IMVIC patterns of + + − − or − + − − as *Escherichia coli* varieties from fecal sources, whereas other members of the coliform group are listed as non-fecal in their commonly designated source. *Aerobacter aerogenes*, the most common isolate in this investigation, is considered to be a common contaminant of plant materials

TABLE 1

Response of isolates from production samples of orange juice to IMVIC and biochemical tests

Types and number	IMVIC tests[1]				Biochemical tests[2]														Motility
	I	M.R.	V.P.	Cit	Bor	Eijk	Nit	Uric	Ura	Gel	Cel	Gly	Inos	Sal	Star	Su	S.R.	H_2S	
Aerobacter aerogenes Types 64 cultures	64 −	64 −	64 +	64 +	64 −	64 −	64 +	64 +	64 +	27 + 37 −	64 +	64 +	63 + 1 −	64 +	64 +	64 +	5 + 59 −	64 −	27 + 37 −
Inter-mediate Types 18 cultures	1 +	1 +	1 −	1 +	1 −	1 −	1 +	1 +	1 −	1 +	1 +	1 +	1 +	1 +	1 +	1 +	1 −	1 −	1 +
	10 −	10 +	10 −	10 +	10 −	10 −	10 +	6 + 4 −	10 −	10 −	10 +	10 +	4 + 6 −	10 +	4 + 6 −	6 + 4 −	10 −	4 + 6 −	10 +
	4 +	4 +	4 +	4 +	4 −	4 −	4 +	4 +	4 −	4 −	4 +	4 +	4 +	4 +	4 +	4 +	4 −	4 −	4 +
	1 +	1 −	1 +	1 +	1 −	1 −	1 +	1 +	1 −	1 −	1 +	1 +	1 +	1 +	1 +	1 +	1 −	1 −	1 +
	2 −	2 +	2 +	2 +	2 −	2 −	2 +	2 +	2 −	1 + 1 −	2 +	2 +	1 + 1 −	2 +	2 +	2 +	2 −	2 −	2 +

[1] I Indol
M.R. Methyl red
V.P. Voges-Proskauer
Cit Citrate

[2] Bor Boric acid lactose 43° C.
Eijk Eijkman-glucose 46° C.
Nit Nitrate
Uric Uric acid
Ura Uracil
Gel Gelatin
Cel Cellobiose
Gly Glycerol
Inos Inositol
Sal Salicin
Star Starch
Su Sucrose
S.R. Soft rot test (Elrod)
H_2S Hydrogen Sulfide

TABLE 2

Response of isolates from experimental samples of orange juice to IMVIC and biochemical tests

Types and number	IMVIC tests[1]				Biochemical tests[2]														
	I	M.R.	V.P.	Cit	Bor	Eijk	Nit	Uric	Ura	Gel	Cel	Gly	Inos	Sal	Star	Su	S.R.	H_2S	Motility
Aerobacter aerogenes Types 33 Cultures	33 −	33 −	33 +	33 +	33 −	33 −	33 +	33 +	33 +	10 + 23 −	33 +	33 +	33 +	33 +	33 +	33 +	4 + 29 −	33 −	10 + 23 −
Intermediate Types 35 Cultures	3 −	3 +	3 −	3 +	3 −	3 −	3 +	3 +	3 −	3 −	3 +	3 +	3 +	3 +	3 +	3 +	3 −	3 −	3 +
	11 +	11 +	11 +	11 +	11 −	11 −	11 +	11 +	11 −	11 −	11 +	11 +	9 + 2 −	11 +	11 +	11 +	11 −	11 −	11 +
	21 −	21 +	21 +	21 +	21 −	21 −	21 +	21 +	21 −	21 −	21 +	21 +	21 +	21 +	21 +	21 +	21 −	21 −	21 +

[1] I Indol
M.R. Methyl red
V.P. Voges-Proskauer
Cit Citrate

[2] Bor Boric acid lactose 43° C.
Eijk Eijkman-glucose 46° C.
Nit Nitrate
Uric Uric acid
Ura Uracil
Gel Gelatin
Cel Cellobiose
Gly Glycerol
Inos Inositol
Sal Salicin
Star Starch
Su Sucrose
S.R. Soft rot test (Elrod)
H_2S Hydrogen Sulfide

and is often referred to as the "grass or grain strain" of the coliform group of organisms.

Hunter *(9)* considered *E. coli* useful as a measure of excretal contamination, but coliform organisms of the genus Aerobacter should not be regarded, except in special instances, as having sufficient significance to be of definite values as indices of pollution. Wolford and Berry *(26)* concluded that routine tests for coliform organisms in orange juice are of limited significance. These investigators stressed the need to supplement the routine tests with special tests to detect members of the fecal *Escherichia coli* group in order to obtain information of value from the public health viewpoint.

Wolford *(25)* and Wolford and Berry *(27)* pointed out that members of the genus Erwinia may confuse the coliform picture when organisms are isolated from plant materials. Elrod *(6)* stated that the manner in which species of Erwinia ferment lactose would tend to classify them as aberrant coliforms. On the basis of their IMVIC patterns, the majority would seem to approximate *Escherichia freundii*. On the other hand, according to their gelatin-liquefying ability, motility and production of acid in glycerol, the relationship seems to be closer to *Aerobacter cloacae*. Stuart and coworkers *(18)* reported that Erwinia resemble the intermediates more than *A. aerobacter* or *E. coli*. In this study the Erwinia, identified mainly because of their ability to produce soft rot, were found to closely resemble *Aerobacter cloacae* in regard to IMVIC pattern, gelatin-liquefying ability, motility and production of acid in glycerol.

SUMMARY

Cultural characteristics of 150 bacteria isolated from orange juice by use of Violet Red Bile agar were studied. When the 150 isolates were grouped according to their IMVIC pattern, 65% were of the Aerobacter type and 35% were of the intermediate type. No cultures of the *Escherichia coli* type were isolated in this investigation.

Identification studies of 17 cultures isolated from one sample of orange juice revealed that five cultures closely resembled *Aerobacter aerogenes*, four, *Escherichia freundii*, two, *Escherichia intermedium* and one, *Serratia marcescens*. The remaining five cultures closely resembled organisms of the genus Erwinia.

Acknowledgment

The authors sincerely appreciate the technical assistance of Miss Ermgard Teutsch, and Dr. B. E. Ellickson's helpful suggestions in preparation of this manuscript.

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TRIOSE COMPOUNDS IN MAPLE SIRUP[a]

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(Manuscript received June 23, 1956)

The characteristic flavor and color of maple sirup are developed during the concentration of the maple sap by atmospheric boiling. An almost flavorless sirup has been produced by low-temperature vacuum evaporation of sap *(9, 22)*. Further, by freeze-drying it is possible to make a completely colorless and flavorless product *(22, 23)*. Therefore, the color and flavor of the sirup is formed by atmospheric boiling from normal constituents of maple sap.

The sap as it comes from the tree contains about 2% solids of which sucrose constitututes more than 98% *(15)*. The remainder of the solids consists of organic acids *(22)*, nitrogenous material *(21)*, salts, and several oligosaccharides *(24)*. Monosaccharides, if the sap remains sterile, are entirely absent. However, fermentation of the sap by contaminating microorganisms will cause inversion of the sucrose to amounts of invert sugar. Work at this laboratory *(24)*, as well as that reported by Edson, Jones, and Carpenter *(7)*, has shown that the growth of microorganisms in sap often results in dark-colored sirup. Hayward and Pederson *(10)* have added acids and alkalies to maple sap, and reported their effect on the sirup produced. During investigations on the color development in sirup *(10, 31)*, it was discovered that the sap passed through an alkaline stage. With the earlier work as a background, the possibility of alkaline degradation of the hexoses was at once suggested as being part of the mechanism of color and flavor development in maple sirup. The alkaline degradation of sugars has been discussed by several authors. Evans in 1942 reported on the breakdown of glucose by alkalies *(8)*. Reviews of color development (browning) in sugar systems have been published by Hodge *(13)* and Liggett and Deitz *(17)*. Sattler and Zerban *(26, 32)* have discussed the chemical fragmentation of the unfermentable reducing substances in molasses. The ultraviolet spectra of sugars in alkaline solution have been studied by Berl and Feazel *(2)*, who found a close relationship among such 3-carbon carbonyls (trioses) as acetol and reductone and the color chromogen of alkaline glucose solutions. Such compounds have been isolated from milk by steam distillation by Keeney and coworkers *(16)*. Also, other carbonyl compounds related to flavor have been isolated by distillation from such diverse food products as cheese *(5)*, corn oil *(4)*, apples, *(12, 14)*, strawberries *(6)*, and pork *(3)*.

Therefore it seemed possible that such carbonyl compounds as trioses could be isolated from U. S. grade AA sirup by steam distillation. Fancy grade maple sirup seemed the most likely source of carbonyl precursors

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[a] Presented at the 16th Annual Meeting of the Institute of Food Technologists, St. Louis, Missouri, June 12, 1956.

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as it is possible to cause a substantial increase in the color and flavor of this grade by the high-flavoring process *(30)*. This paper presents evidence that trioses (3-carbon carbonyl compounds) are present in maple sirup and could be related to the alkaline breakdown of hexoses.

EXPERIMENTAL

Isolation of carbonyl compounds. A 200-g. sample of fancy grade maple sirup was steam distilled into 50 ml. of a solution containing 250 mg. of 2,4-dinitrophenylhydrazine[c] (crystallized from benzene) first dissolved in 15 ml. of concentrated H_2SO_4. Three 200-ml. fractions were collected from the sirup. Two blanks were run, one before and one after the sample, using the same volume of water and steam. The 5 distillates were transferred to 250-ml. beakers, stirred, covered, and allowed to stand at 70°-75° F. overnight. The blanks did not contain 2,4 DNPH-precipitable material, but the first 200-ml. distillate contained a quantity of orange, amorphous precipitate. The other two distillates contained only a trace of precipitate and were discarded. The precipitate of distillate one was collected by filtration through a fritted glass disc by aspiration, washed with dilute sulfuric acid and a small quantity of distilled water, and dried in a vacuum at 50° C. Five-and-a-half milligrams of the dried mixture of 2,4 DNPH derivatives were obtained from the 200 g. of sirup.

To determine roughly the type and amount of carbonyl compounds present, the dried mixture was treated with 10-ml. portions of petroleum ether (Skellysolve B,[d] b.p. 63-70° C.), filtering off the solvent between each addition. This washing was repeated until the petroleum ether dissolved no more material. Then absolute ethanol was substituted for petroleum ether. Fractions dissolved by petroleum ether and by ethanol (solutions A and B) contained the monocarbonyl derivatives. The residue on the filter was then treated with chloroform to isolate the dicarbonyl derivatives (solution C). The small, final residue on the filter (Solution D) was soluble in ethyl acetate. While these solubility separations (Figure 1) furnished information as to the type of carbonyls present, it was later found that they served no particular purpose in the subsequent quantitative separation of the 2,4 DNPH derivatives in the original crude mixture.

Chromatographic separation of the 2,4 DNPH derivatives. After testing several types of chromatographic materials reported in the literature (4, 5, 6, 12, 25, 27, 29) for the columnar separation 2,4 DNPH derivatives, silicic acid was found best suited for separating the particular compounds isolated from the maple sirup. The crude precipitate was dissolved in chloroform and silicic acid was added, equivalent to about five times the weight of sample to form a slurry. This was evaporated to dryness at room temperature to precipitate the 2,4 DNPHs onto the acid. A silicic acid chromatographic column, 22 mm. x 15 cm., was prepared by adding a slurry of the material in petroleum ether (b.p. 63°-70°C.) to a tube 22 mm. ID x 40 cm. and packing it under two pounds of air pressure. The dry 2,4 DNPH-silicic acid mixture was transferred to the column with Skellysolve B. To effect a complete separation and elution of the derivatives, solvent mixtures were used starting with 5% diethyl ether in Skellysolve B. Several bands were formed from the orange mass at the top of the column. The polarity of the solvent was then raised by increasing the percentage of diethyl ether until 100% diethyl ether was being used. This was followed by a series of ethanol in diethyl ether in which the percentage of ethanol was increased from 25% to 100%. Finally the remaining bands were eluted with chloroform. From the elution of the developed bands 8 solvent fractions were obtained. The elution solvent, peak effluent volume, and color in alkaline ethanol are recorded for separated fractions in Table 1.

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[c] Hereafter designated as 2,4 DNPH.

[d] Mention of specific commercial materials and equipment throughout this paper does not constitute recommendation for their use above similar materials and equipment of equal quality.

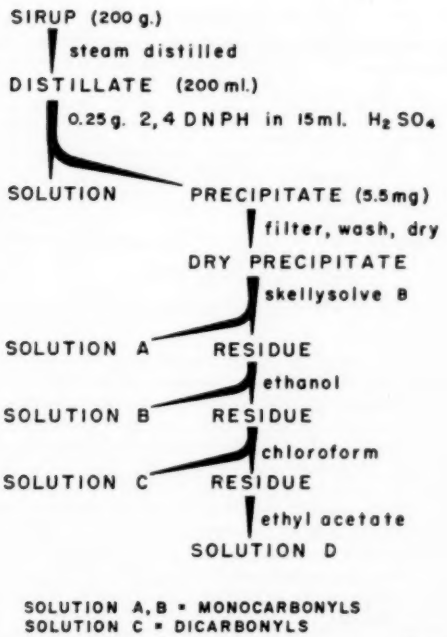

Figure 1. Flow sheet of the solvent separation of the 2,4-dinitrophenylhydrazine derivatives of the steam distillable carbonyl compounds in maple sirup.

Identification of the 2,4 DNPH derivatives. A search of the literature failed to provide a very complete characterization of the 2,4 DNPH derivatives of carbonyl compounds of the type expected to be formed in maple sirup. Therefore, the 2,4 DNPH derivatives of a number of the carbonyl compounds related to possible alkaline sugar degradation products were prepared and carefully purified for the determination of such physical measurements as solubilities, melting points, spectral absorption curves and elemental chemical analyses. Details of the preparation, purification, and determination of the chemical and physical characteristics of these compounds will be described in another paper. Table 2 presents the physical data of a selected number of known compounds and those isolated from maple sirup. Only known compounds that have similarity to those obtained from maple sirup have been included.

TABLE 1

Data on the silicic acid column separation of 2,4 DNPH derivatives from maple sirup

Fraction	Eluting solvent	Peak effluent volume ml.	Color in alkaline methanol
1	10% diethyl ether in Skellysolve B	540-555	red
2	10% diethyl ether in Skellysolve B	710-800	red
3	15% diethyl ether in Skellysolve B	1650-1700	red
4	25% diethyl ether in Skellysolve B	1725-1765	red
5	100% diethyl ether	1780-2080	violet
6	50% diethyl ether in ethanol	2090-2140	violet
7	100% ethanol	2145-2205	violet
8	100% chloroform	2225-2500	violet

TABLE 2

Physical data on known and unknown 2,4-dinitrophenylhydrazine derivatives

Compound	Color in alkaline methanol	Melting point		Absorption solvent	Maxima λ
		Found ° C. (corr.)	Cited ° C.		
Acetol	Violet	295-298	296-97	(16) Dioxane	394,434
Methyl glyoxal	Violet	304-305	299-300	(11) Methanol	357,395
Glycerylaldehyde	Violet	298-299	301(19)	Methanol	224,406,430
Reductone	Violet	. . .	. . .	Methanol	402,436
Hydroxymethyl furfural	Red	186	. . .	Methanol	384,299,262
Fraction 2	Red	104-106	. . .	Methanol	225,360
Fraction 5	Violet	177-185	. . .	Methanol	216,400
Fraction 8	Violet	296-299	. . .	Dioxane	394,434

Of the 8 fractions obtained from the chromatographic separation, only fractions 2, 5, and 8 contained sufficient material for the determination of their physical constants. These 3 isolates were evaporated to dryness at room temperature, and then completely dried at 50°C. in a vacuum oven. Weighed portions of the dry materials of fractions 2 and 5 were dissolved in dry methanol. Because of the low solubility of fraction 8 in methanol, 1,4-dioxane was used for this material to prepare a solution of sufficient concentration to make spectral photometric measurements. The absorption measurements from 225 mμ to 550 mμ were made on these 3 samples, using a Cary recording spectrophotometer. These measurements were compared with those of known 2,4 DNPH derivatives. The remaining materials of the 3 fractions were crystallized from acetone-water solution. Enough crystalline compound was obtained for melting point determinations. Data on the 3 fractions are listed in Table 2 along with similar data from known compounds.

RESULTS AND DISCUSSION

The largest portion of the steam distillable material from the maple sirup proved to be carbonyl compounds similar to one or more of the 3-carbon carbonyl compounds; glycerylaldehyde, reductone, methyl glyoxal, and acetol. Fractions 5 and 8 from the chromatographic column consti-

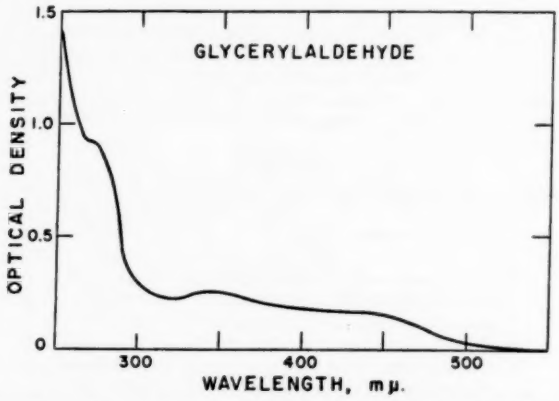

Figure 2. Absorption curve of the 2,4-dinitrophenylhydrazine derivative of glyceryl-aldehyde in methanol.

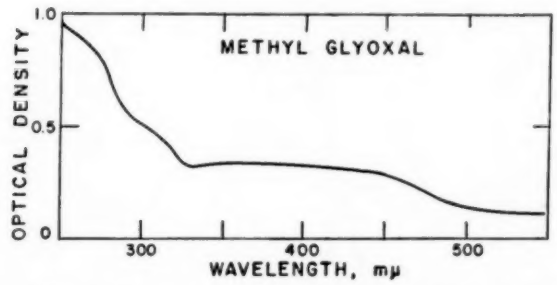

Figure 3. Absorption curve of the 2,4-dinitrophenylhydrazine derivative of methyl glyoxal in methanol.

tuted about 75% of the total amount of material in the steam distillate. Both fractions as shown by their violet color in alkaline methanol *(19, 20, 27)* as well as by their absorption curves (Figures 2, 3, 4, 5, and 6) were an osazone type of 2,4 DNPH derivative such as obtained from dicarbonyl and α-hydroxy carbonyl compounds *(18, 28)*. The melting point of the crystalline compounds constituting fraction 8 corresponded to those obtained in this laboratory for the osazone 2,4 DNPH derivatives of purified methyl glyoxal, acetol, and glycerylaldehyde and also agreed with the melting points cited in the literature for these compounds *(1, 16, 19)*. No reference was found for the melting point of any 2,4 DNPH derivative of reductone, and the authors have yet to prepare a good crystalline 2,4 DNPH derivative of this compound. This is probably due to the many possible reaction products that could be obtained from a solution of reductone and 2,4 DNPH. However, the absorption curve of acetol in dioxane was almost identical with that of fraction 8 in the same solvent (Figure 7). The very low solubility of the acetol osazone in methanol required the use of dioxane as the solvent for its absorption curve. This was also true for

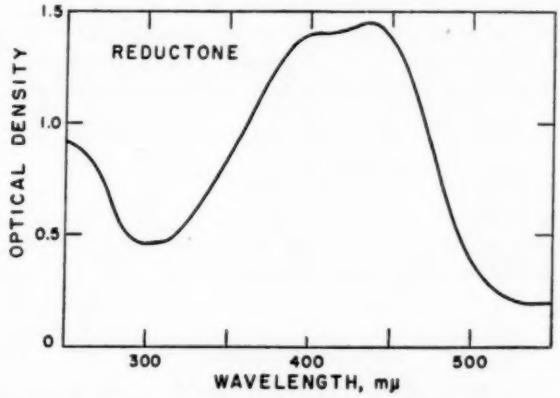

Figure 4. Absorption curve of the 2,4-dinitrophenylhydrazine derivative of reductone in methanol.

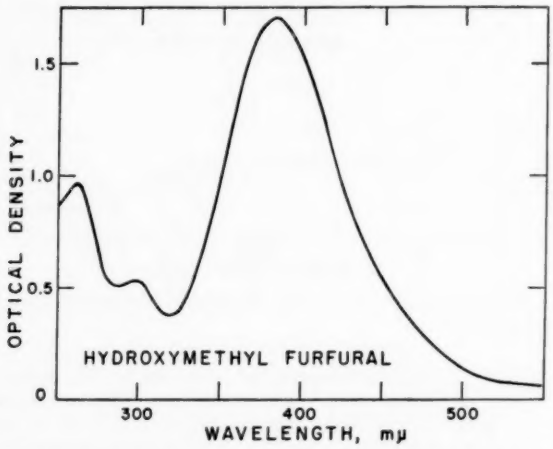

Figure 5. Absorption curve of the 2,4-dinitrophenylhydrazine derivative of hydroxymethylfurfural in methanol.

the material in fraction 8. This difference in the solubilities of the derivatives of the four 3-carbon carbonyls (trioses) favors the possibility that fraction 8 is acetol.

Regarding fraction 5, all of the physical data collectively indicate that this isolate was not a pure compound. Although the absorption curve of the fraction in methanol resembled the absorption curves of the osazones of reductone and glycerylaldehyde in the same solvent, the melting point of the material crystallized from acetone-water was indefinite and much too low for the osazone of any of these compounds. However, the material of fraction 5 showed a closer relationship by its spectral absorption curve to the triose type compound than to other types of compounds sometimes involved in sugar and carbohydrate degradation such as the furfurals, pyruvic acid, and simple monocarbonyls as acetaldehyde or acetone.

Fraction 2 gave a few very well-formed crystals which melted at 104°-106° C. The alcoholic KOH test indicated a monocarbonyl derivative and

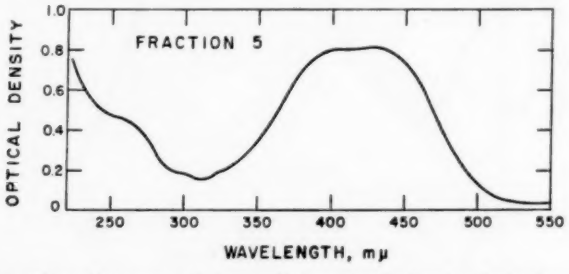

Figure 6. Absorption curve of the 2,4-dinitrophenylhydrazine derivative of fraction 5 in methanol.

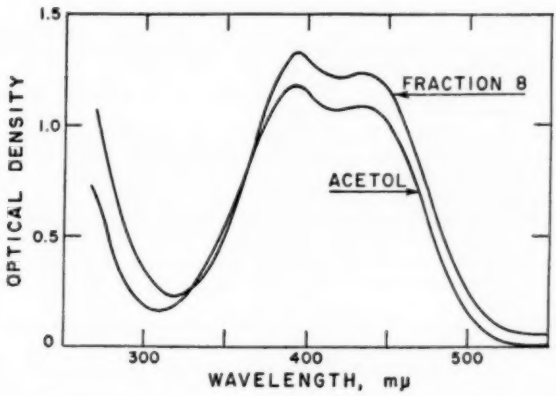

Figure 7. Absorption curves of the 2,4-dinitrophenylhydrazine derivatives of acetol and fraction 8 in dioxane.

the absorption curve with MeOH as solvent was also characteristic of a 2,4-dinitrophenylhydrazone. However, none of the 2,4 DNPH derivatives prepared by the authors from known carbonyls was found to compare closely with fraction 2. Examination of the literature showed that Allen *(1)* reported melting points for 2,4 DNPH derivatives of saturated, straight chain aldehydes C_7, C_{10}, and C_{11} between 103° and 106° C. Buss and MacKinney *(4)* report the recovery, by steam distillation of rancid corn oil, of *n*-undecanal whose 2,4 DNPH derivative melted at 104°C. and whose absorption curve gave characteristics identical with fraction 2. Presence of this type of carbonyl compound in maple sirup was not foreseen theoretically. Its exact identity may furnish ideas for other mechanisms of flavor or color formation in maple sirup. Larger scale isolations of these carbonyl compounds by steam distillation and by several organic solvents are now under way.

Demonstration of the presence of triose carbonyl compounds in maple sirup suggests that they are intermediate in color and flavor development. These very reactive compounds through polymerization can form chromogens, and by interaction with other substances could form flavor bodies.

SUMMARY

Presence of trioses has been demonstrated in the steam distillate of maple sirup. The major portion of these compounds has been identified as probably acetol. These active carbonyl compounds are probable precursors of maple flavor and color.

Acknowledgment

The authors wish to express their thanks to Anne M. Smith of the Analytical, Physical Chemical, and Physics Section of the laboratory for the spectrophotometric measurements.

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NOTES AND LETTERS

ADDITIONAL TABLES FOR A METHOD OF CHOOSING JUDGES FOR A SENSORY EXPERIMENT

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(Manuscript received July 2, 1956)

Additional tables for a method of choosing a panel of judges for a sensory experiment to insure that the individual judges can detect differences at any given probability are given in this article. Use is made of the results obtained by Chapman *(1)* to calculate the probability of obtaining exactly s correct matchings and s or more correct matchings when two series of different lengths are matched.

The author has discussed *(2)* how judges may be chosen to insure that they can detect differences at a given probability level when the potential judges are asked to match two series of t elements each. In many instances, especially in taste testing experiments, judges are expected to be able to discriminate between very slight differences. If taste fatigue is a problem, a potential judge will not be able to taste too many samples in the screening. Then the method discussed in the author's previous paper may not be as efficient as the present method.

Chapman gives the following formula for the probability of obtaining exactly s correct matchings when two series of t and u elements each are matched. He assumes $t \leq u$ and that exactly t elements of the u series are true apposites of the t series.

$$P(s) = \frac{t!}{s!\,u!}\left[\frac{(u-s)!}{0!\,(t-s)!} - \frac{(u-s-1)!}{1!\,(t-s-1)!} + \ldots + (-1)^{t-s}\frac{(u-t)!}{(t-s)!\,0!}\right] \quad \text{(i)}$$

He also gives the following formula for the probability of obtaining s or more correct matchings.

$$P(s \text{ or more}) = P(s) + P(s+1) + \ldots + P(t) \quad \text{(ii)}$$

Tables have been computed using (i) and (ii) for $u = (t+1)$ and $u = (t+2)$ up to $u = 10$. Since the probabilities converge so rapidly and because of fatigue, it is not anticipated that further tables will be needed; but any worker can extend the tables in any particular case with a table of factorials and reciprocals. These tables are given at the end of this article.

Let us assume that we require judges for an experiment to determine if there is any difference in the saltiness of meat cured by five different processes at say the 5% level. Further, let us assume that the maximum number of samples a potential judge can taste without fatigue is ten. Also it is anticipated that the results of two or three of the treatments will be very close together. If two treatments are expected to produce very similar results, we would prepare 4 solutions of salt varying in concentration in duplicate. Then another solution would be prepared to have

a concentration half-way between two of the solutions in duplicate. The potential judges would then be asked to match the two series of solutions, one series having four and the other five. From Table 2 it is seen that in order to be a judge a person would have to correctly match three or more samples. If five solutions were made in duplicate, a judge would be required to match correctly four or more samples before he was acceptable.

This method may be used to choose judges if it is impossible to prepare all samples in duplicate or if some of the duplicates are lost for one reason or another.

TABLE 1

Probability of obtaining exactly s correct matching (u = t + 1)

t	u	s → 0	1	2	3	4	5	6	7	8	9
1	2	.5000	.5000								
2	3	.5000	.3333	.1667							
3	4	.4583	.3750	.1250	.0417						
4	5	.4417	.3667	.1500	.0333	.0083					
5	6	.4292	.3680	.1528	.0417	.0069	.0014				
6	7	.4204	.3679	.1577	.0437	.0089	.0012	.0002			
7	8	.4139	.3679	.1609	.0460	.0095	.0016	.0002	.0000		
8	9	.4088	.3679	.1635	.0477	.0102	.0017	.0002	.0000	.0000	
9	10	.4047	.3679	.1656	.0491	.0107	.0018	.0002	.0000	.0000	.0000

TABLE 2

Probability of obtaining s or more correct matchings (u = t + 1)

t	u	s → 0	1	2	3	4	5	6	7	8	9
1	2	1.0000	.5000								
2	3	1.0000	.5000	.1667							
3	4	1.0000	.5417	.1667	.0417						
4	5	1.0000	.5583	.1916	.0416	.0083					
5	6	1.0000	.5708	.2028	.0500	.0083	.0014				
6	7	1.0000	.5796	.2117	.0540	.0103	.0014	.0002			
7	8	1.0000	.5861	.2182	.0573	.0113	.0018	.0002	.0000		
8	9	1.0000	.5912	.2233	.0598	.0121	.0019	.0002	.0000	.0000	
9	10	1.0000	.5953	.2274	.0618	.0127	.0020	.0002	.0000	.0000	.0000

TABLE 3

Probability of obtaining exactly s correct matchings (u = t + 2)

t	u	s → 0	1	2	3	4	5	6	7	8
1	3	.6667	.3333							
2	4	.5833	.3333	.0833						
3	5	.5333	.3500	.1000	.0167					
4	6	.5028	.3555	.1167	.0222	.0028				
5	7	.4817	.3591	.1270	.0278	.0040	.0004			
6	8	.4664	.3613	.1347	.0317	.0052	.0006	.0000		
7	9	.4547	.3628	.1405	.0349	.0062	.0008	.0001	.0000	
8	10	.4455	.3638	.1451	.0375	.0070	.0010	.0001	.0000	.0000

TABLE 4

Probability of obtaining s or more correct matchings (u = t + 2)

t	u	s → 0	1	2	3	4	5	6	7	8
1	3	1.0000	.3333							
2	4	1.0000	.4166	.0833						
3	5	1.0000	.4667	.1167	.0167					
4	6	1.0000	.4972	.1417	.0250	.0028				
5	7	1.0000	.5183	.1592	.0322	.0044	.0004			
6	8	1.0000	.5335	.1722	.0375	.0058	.0006	.0000		
7	9	1.0000	.5453	.1825	.0420	.0071	.0009	.0001	.0000	
8	10	1.0000	.5545	.1907	.0456	.0081	.0011	.0001	.0000	.0000

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HISTOLOGICAL NOTE ON A FROZEN SNAP BEAN PROBLEM

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A quality problem that occurs in frozen snap beans is the sloughing of the skin of the cooked, ready-to-serve product. This phenomenon is not so apparent in either canned or freshly cooked beans but consumer acceptance of the frozen product is adversely affected. Singleton *(3)* has discussed the skin sloughing problem, among others, and reported variations of time and temperature of blanch not to be successful in solving the problem. He did find a correlation between cavities around the seed and sloughing of the outer skin after freezing. Some varieties, which have such cavities only during the early stages of maturity, sloughed their skins during these stages. Singleton's discussion further indicates that considerable varietal difference exists with respect to this characteristic. Enzie *(1)* has pointed out that some varieties slough their skins more readily than others, a factor of importance in evaluating new varieties.

A microscopic study was made to determine where the break which results in the skin sloughing occurs. A sample of fresh market beans was used. One lot was deaerated in formaldehyde acetic acid, ethyl alcohol fixative. Another lot was frozen at –18° C. (0° F.) after slicing and steam blanching for 2 minutes in the usual manner. After storage for a few weeks a portion of these frozen beans was thawed, deaerated, and

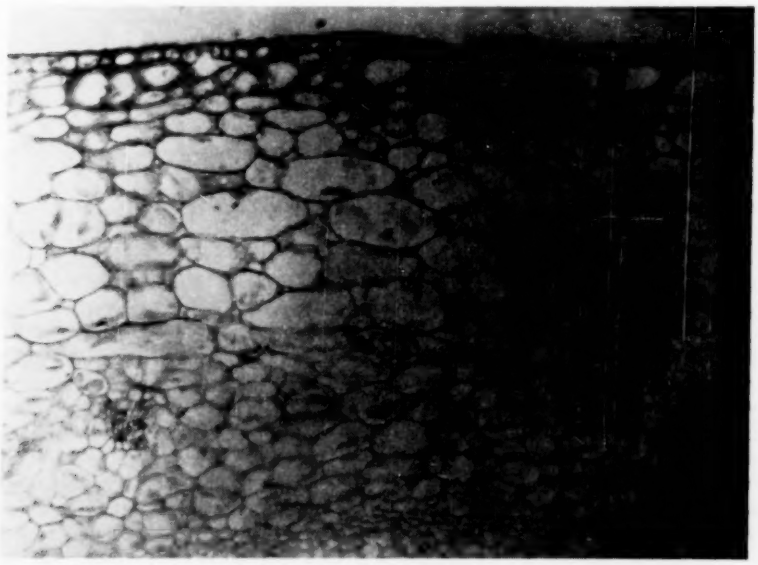

Figure 1. Transverse section of mature fresh snap bean. Approx. 107×.

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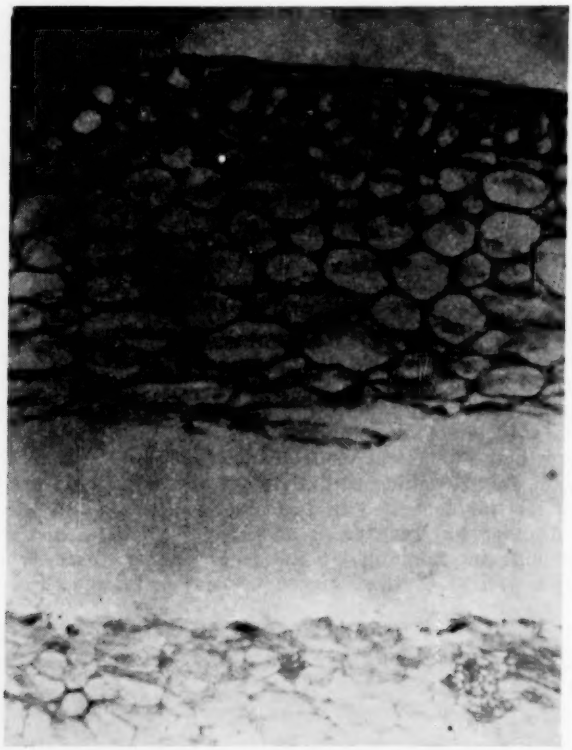

Figure 2. Transverse section of frozen and thawed snap bean. Approx. 107×.

fixed as the fresh ones had been. Another portion of the same frozen beans was cooked for 12 minutes in boiling water and then similarly fixed. All samples were then dehydrated in alcohol, infiltrated with xylene and paraffin and sectioned, mounted, and stained with Heidenhain's hematoxylin according to the standard procedures described by Johansen *(2)*.

The results are clearly shown in the accompanying photomicrographs. Figure 1, the fresh bean, shows the normal structure of the mature snap bean in transverse section. There is a single epidermal layer with small cells and rather heavily staining walls. Below this are two clearly differentiated rows of hypodermal cells with thick walls. Further below are about 10 rows of large thin-walled parenchyma cells before any vascular cells can be seen. The latter are in the deeper layers of cells which are smaller in size again. Thus, there are 4 distinct layers. Figure 2 shows where the break occurs after freezing and thawing; namely, in the region of the largest thin-walled parenchyma cells just outside the vascular region. Figure 3, the cooked frozen product, shows the same break, the only difference being that it occurred in about the middle of this large-celled region in this particular specimen. Cooking caused the cells to shrink a little.

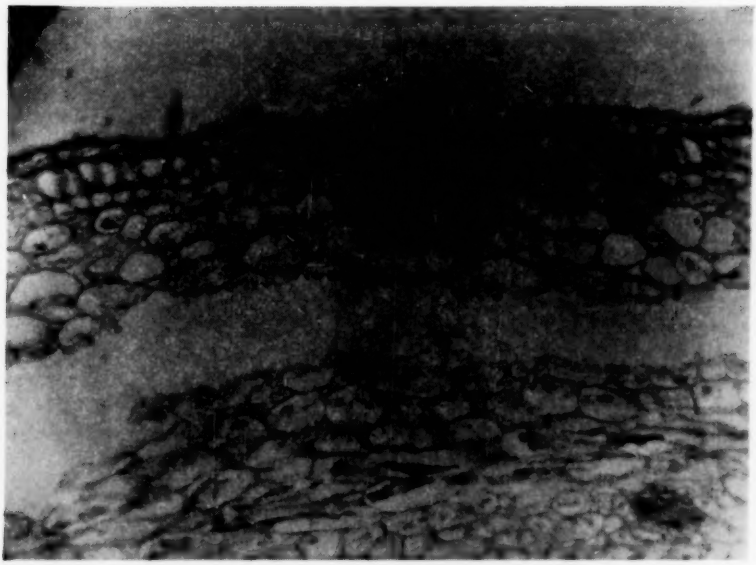

Figure 3. Transverse section of cooked frozen snap bean. Approx. 107×.

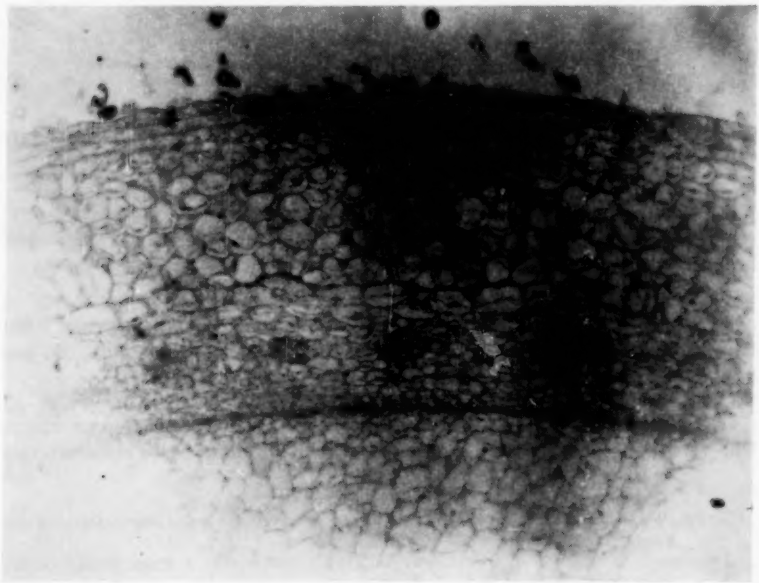

Figure 4. Transverse section of very young snap bean, just after blossom drop. Approx. 107×.

Thus the photomicrographs clearly depict what happens as a result of processing to cause the loosening of the upper tissue layers of the bean.

Figure 4 presents a transverse section of a very young snap bean just after blossom drop. It was prepared in the same way as the other photomicrographs except that it was stained with safranin and light green. This figure shows that all the future tissue regions were blocked out at this early stage of development. The chief effect of maturation is increase in cell size and loss of some of the epidermal hairs. The large-celled parenchyma layer where the break occurs after freezing, is already easily recognizable. The lowest layers do not all appear on the previous figures, but in this figure the vascular regions can be clearly recognized. Figure 5

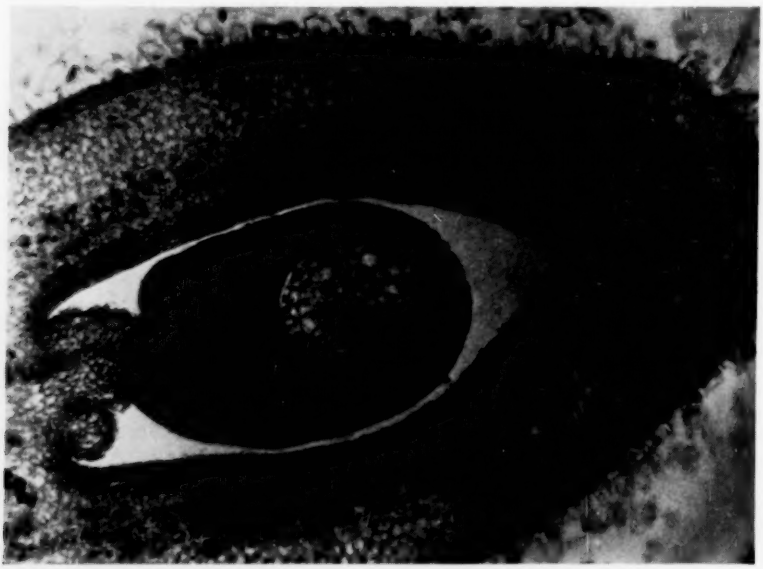

Figure 5. Transverse section of ovary of snap bean blossom. Approx. 107×.

presents a cross section of a bean ovary at blossom time. The ovule, which develops into the seed, and the ovary wall, which develops into the pod, are clearly visible at this early stage.

This series of photomicrographs shows that the break occurs in the layer of large thin-walled parenchyma cells outside the vascular region. The tissue regions involved are delineated at or before the blossom stage.

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2. Johansen, D. A. *Plant Microtechnique*. 1941. McGraw-Hill Book Company, New York, N. Y.
3. Singleton, Gray. Bean varieties for freezing. *Quick Frozen Foods*, 13 (6) 51-53. 1953.

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